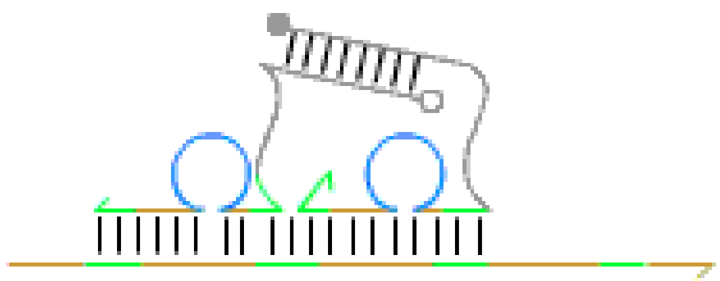
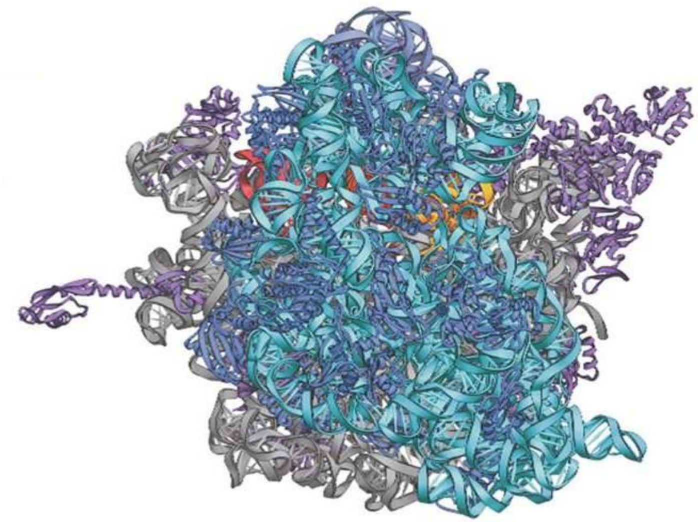
Programming Dynamic DNA Nanosystems

Andrew Turberfield
Department of Physics
University of Oxford





EPSRC
Engineering and Physical Sciences Research Council
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Building with biomolecules: synthetic biology from the bottom up

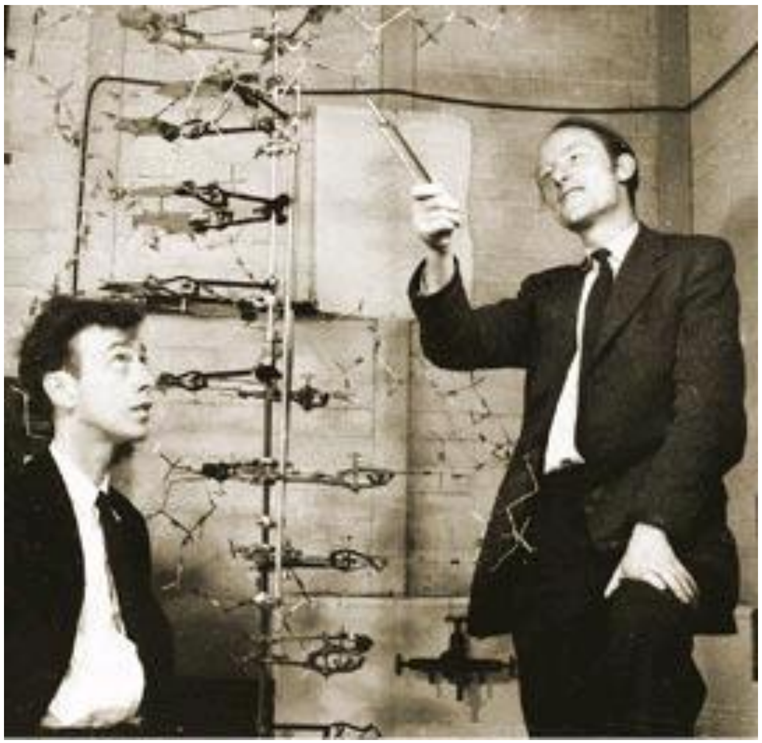
Given the ability to manufacture 3D structures with nm precision, and to create molecular systems that integrate sensing, computation and actuation -

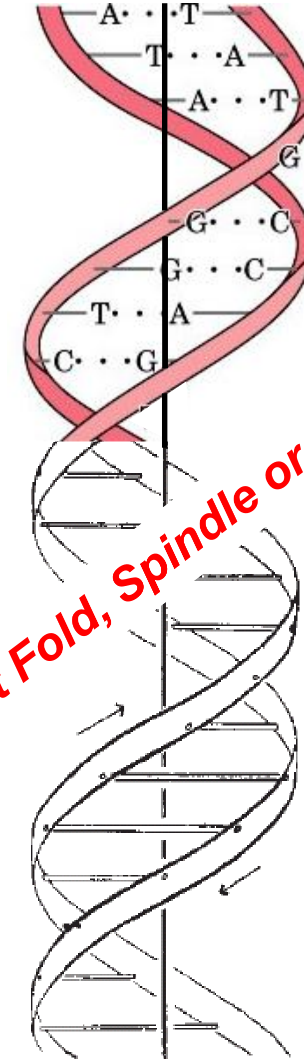
what should we make?

Introduction

DNA Nanostructures:
building and computing with
DNA helices and junctions

DNA: an unlikely material for building devices and executing programs?

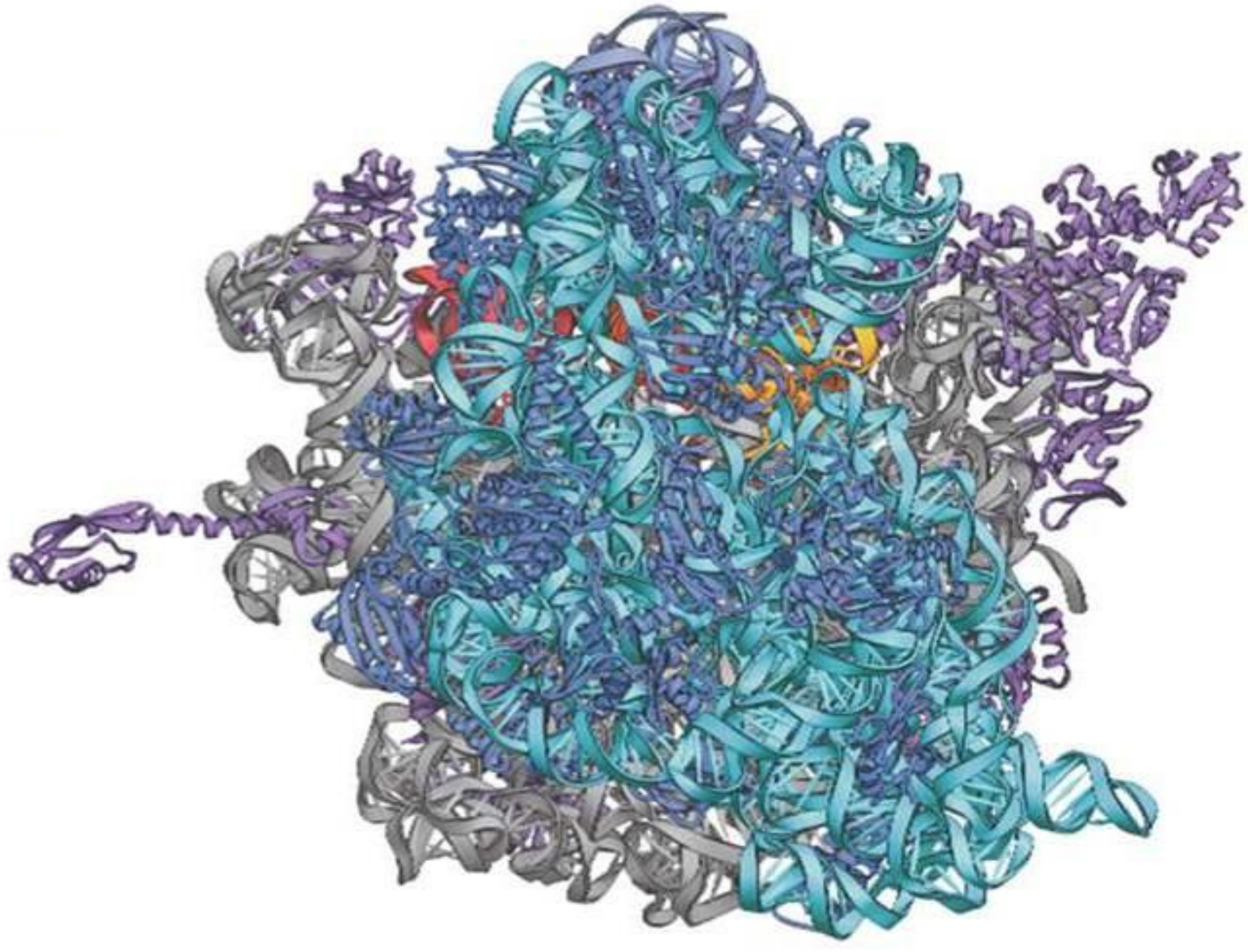




Do Not Fold, Spindle or Mutilate

J. D. Watson and F. H. C. Crick, *Nature* **171**, 737 (1953)

Not so unlikely: all life depends on nucleic acid structures and machines



X-ray structure of T. thermophilus ribozome

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Juan Jin
Behnam Najafi
Emma Silvester
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Sam Tusk
Katie Young

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Helen Carstairs
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Russell Goodman
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Natalie Haley
Céline Journot
Parminder Lally
Le Liang
Alex Lucas
James Mitchell
Robert Machinek
Mireya McKee **Richard Muscat**
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Ibon Santiago Gonzales
Thomas Sharp
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Collaborators

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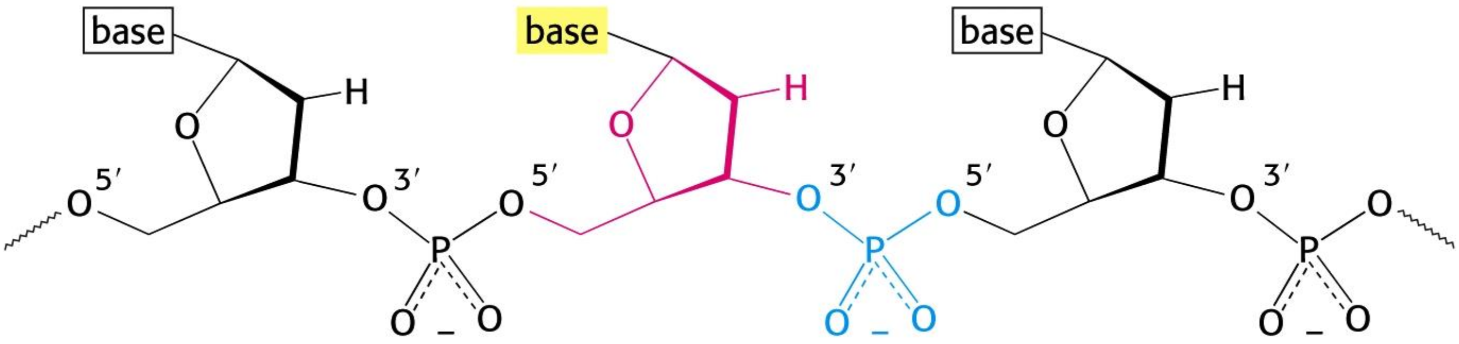
Marta Kwiatkowska	**Computer Science, Oxford**
Tom Ouldridge	**Imperial College London**
Hiroshi Sugiyama **Masayuki Endo**	iCeMS, Kyoto University
Rachel O’Reilly **Phillip Milnes** **Tom Wilks**	Chemistry, Warwick University
Rob Cross	Warwick Medical School

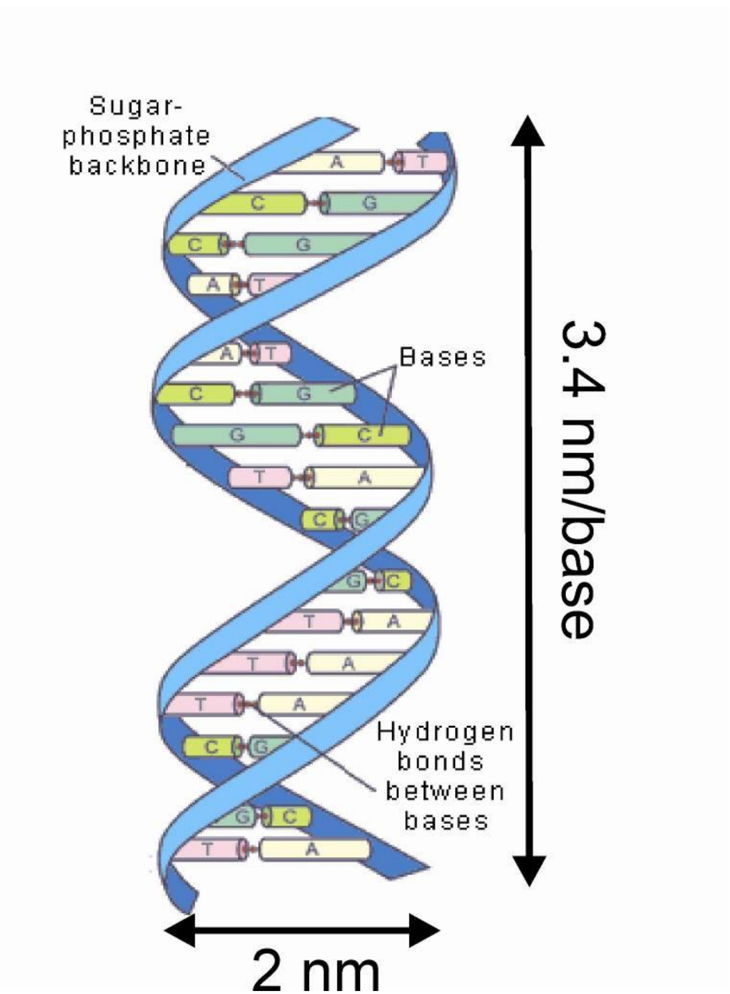
DNA

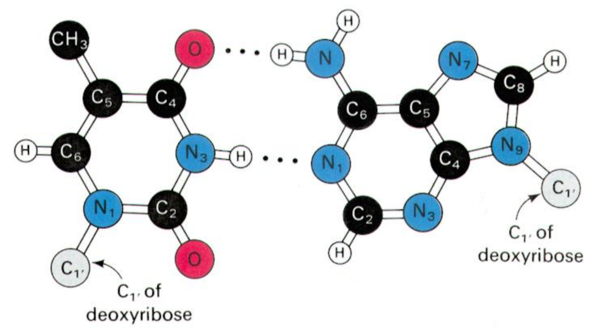


Information storage with an alphabet of four characters:

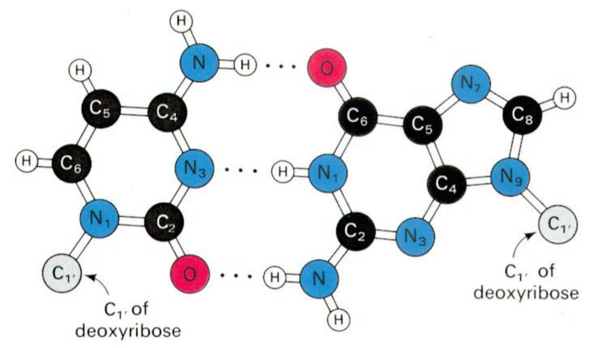
base = A, C, G or T

DNA


Sugar-
phosphate
backbone
Bases
Hydrogen
bonds
between
bases
3.4 nm/base
2 nm


CH3
C1' of
deoxyribose
C1' of
deoxyribose

T ——— A

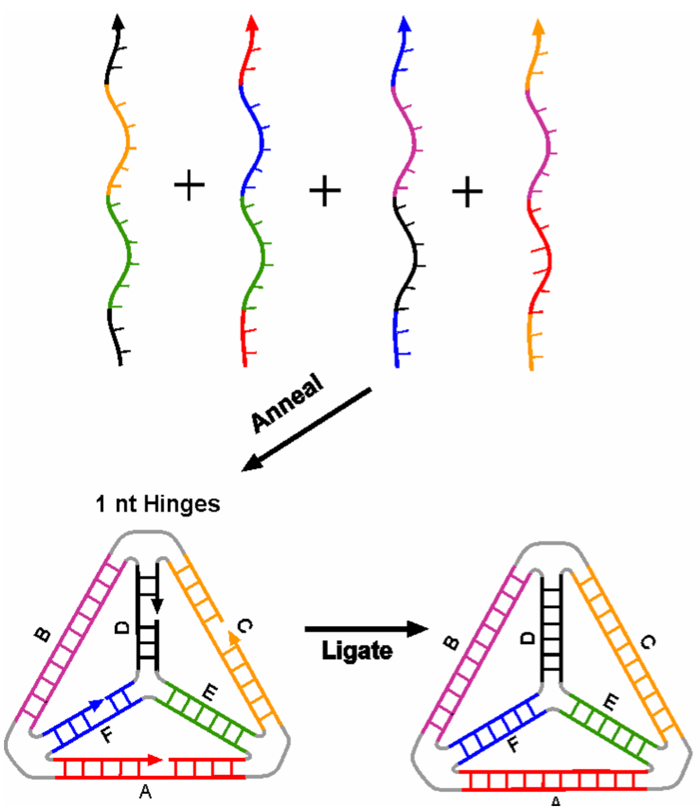

C1' of
deoxyribose
C1' of
deoxyribose

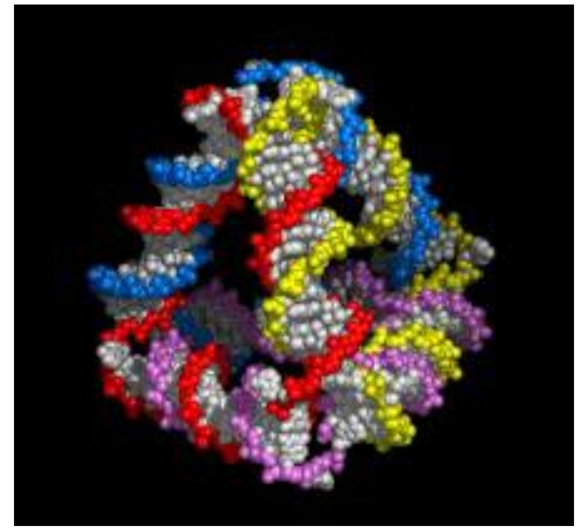
C ——— G

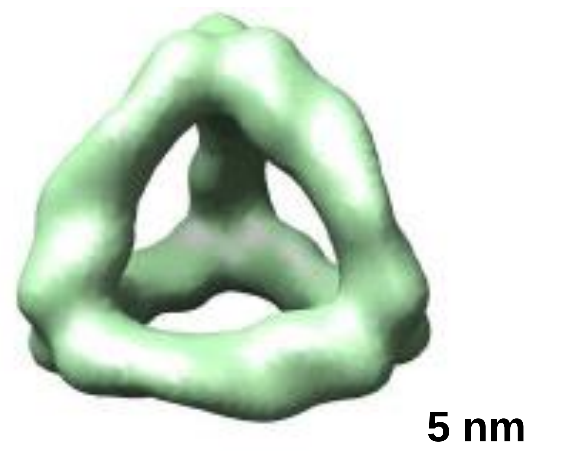
Rapid Chiral Assembly of Rigid DNA Building Blocks for Molecular Nanofabrication

R. P. Goodman,[1] I. A. T. Schaap,[2] C. F. Tardin,[2] C. M. Erben,[1] R. M. Berry,[1] C. F. Schmidt,[2] A. J. Turberfield[1]*

Science **310**, 1661-1665 (2005)

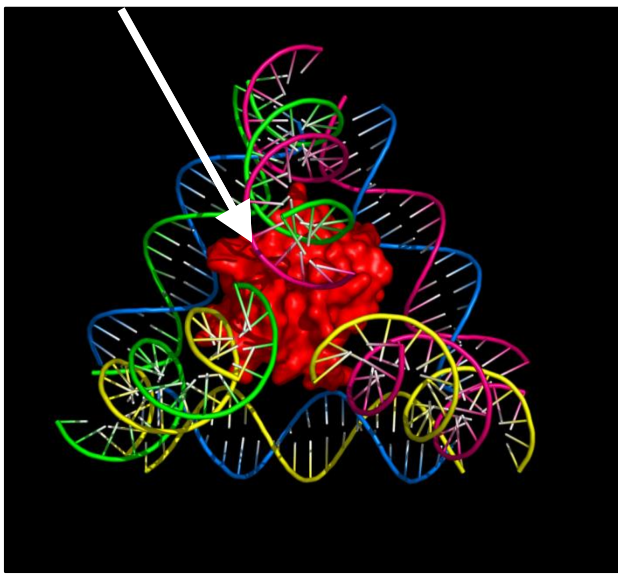




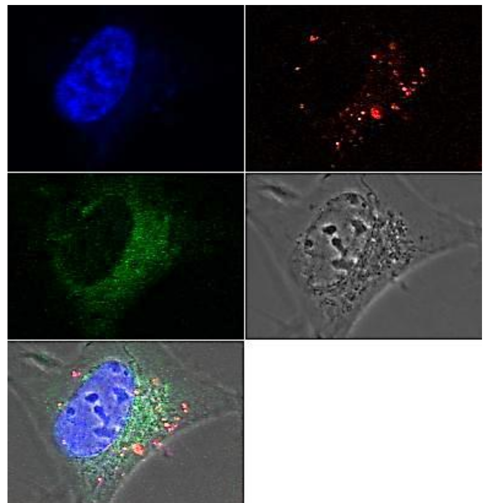


A molecular cage

Cytochrome *c*



Single-Molecule Protein Encapsulation in a Rigid DNA Cage
C. M. Erben, R. P. Goodman, A. J Turberfield
Angew. Chem. Int. Ed. **45**, 7414-7417 (2006).

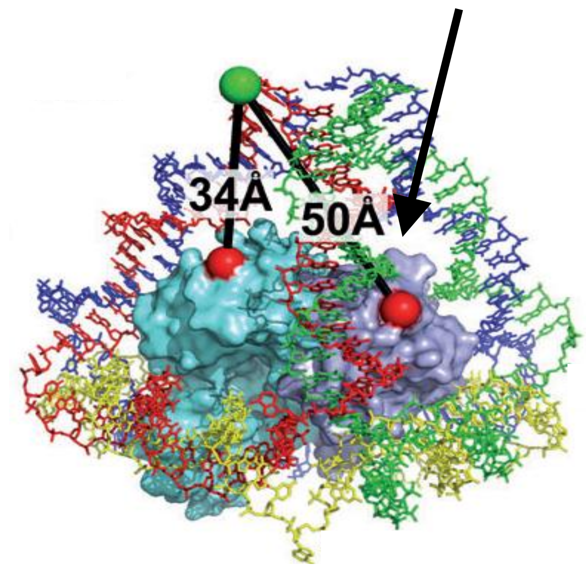


Red: Cy5 (tetrahedron)
Blue: nuclear stain
Green: LysoSensor™ (lysosomes)
Grey: phase contrast.

Scale bar 20μm

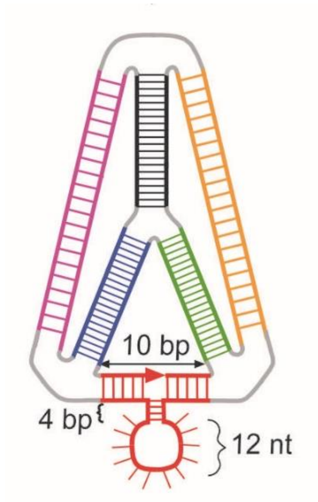
DNA cage delivery to mammalian cells
A.S. Walsh, H.F. Yin, C.M. Erben, M.J.A. Wood, A.J. Turberfield
ACS Nano 5, 5427-5432 (2011)

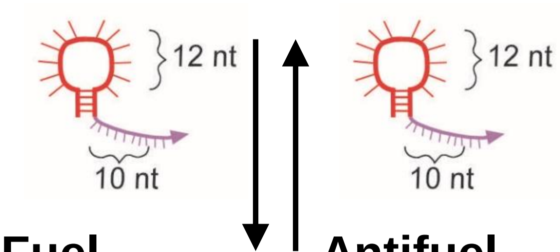
Catabolite Activator Protein



Non-covalent single transcription factor encapsulation inside a DNA cage
R. Crawford, C. M. Erben, J. Periz, L. M. Hall, T. Brown, A. J. Turberfield, A. N. Kapanidis
Angew. Chem. Int. Ed. **52**, 2284-2288 (2013)

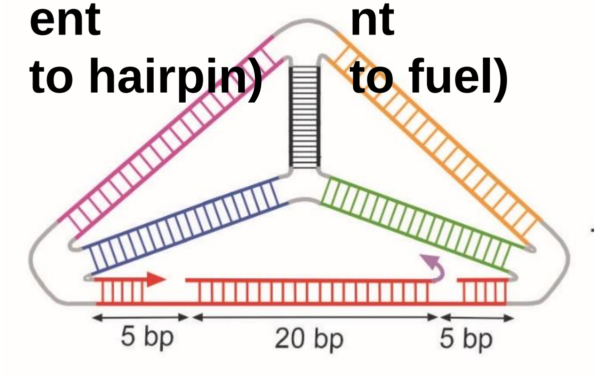
Controlled cage opening

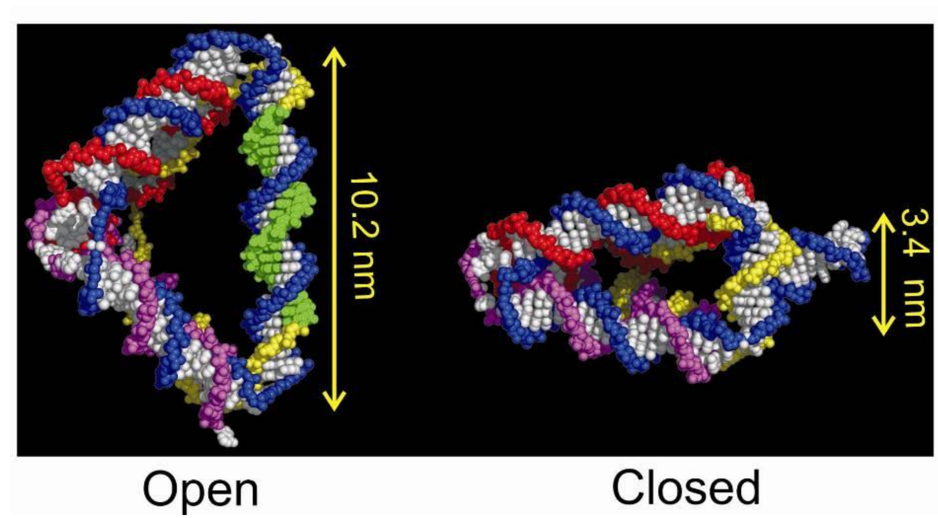




Fuel (complement to hairpin)

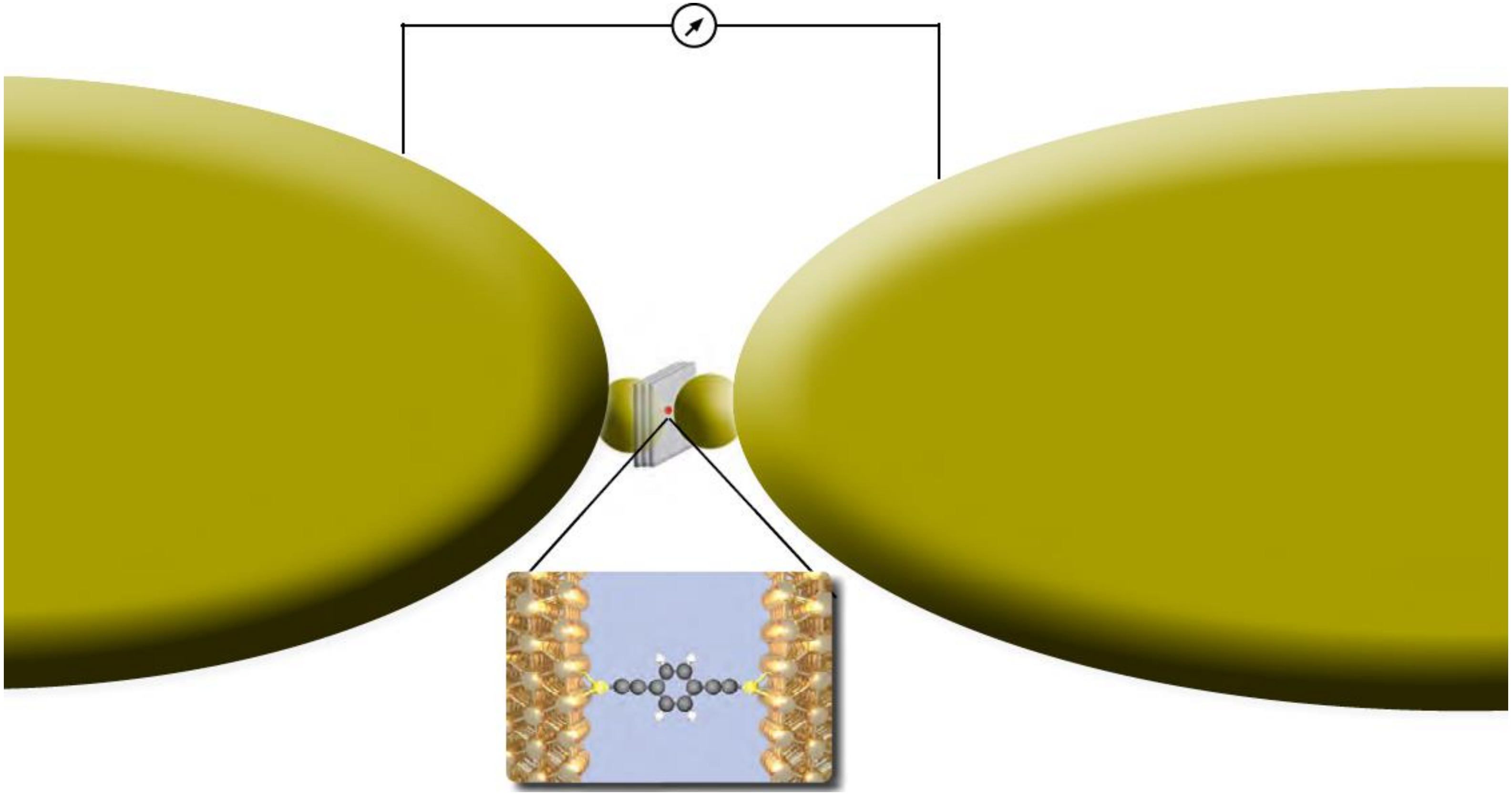
Antifuel (complement to fuel)

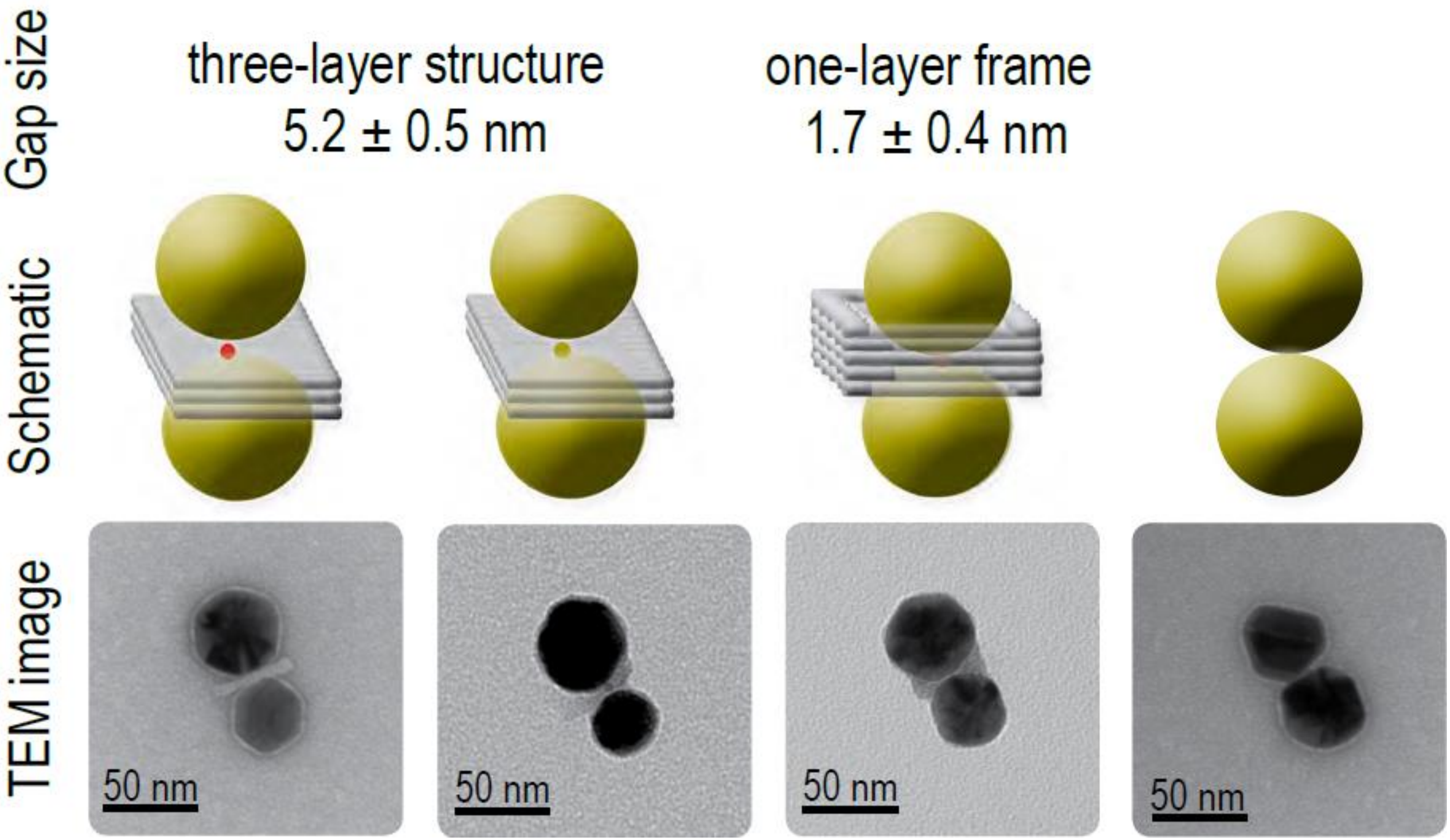




R. P. Goodman, M. Heilemann, S. Doose, C.M. Erben, A.N. Kapanidis, A. J Turberfield *Nature Nanotech.* **3**, 93-96 (2008)

Molecular electronics?

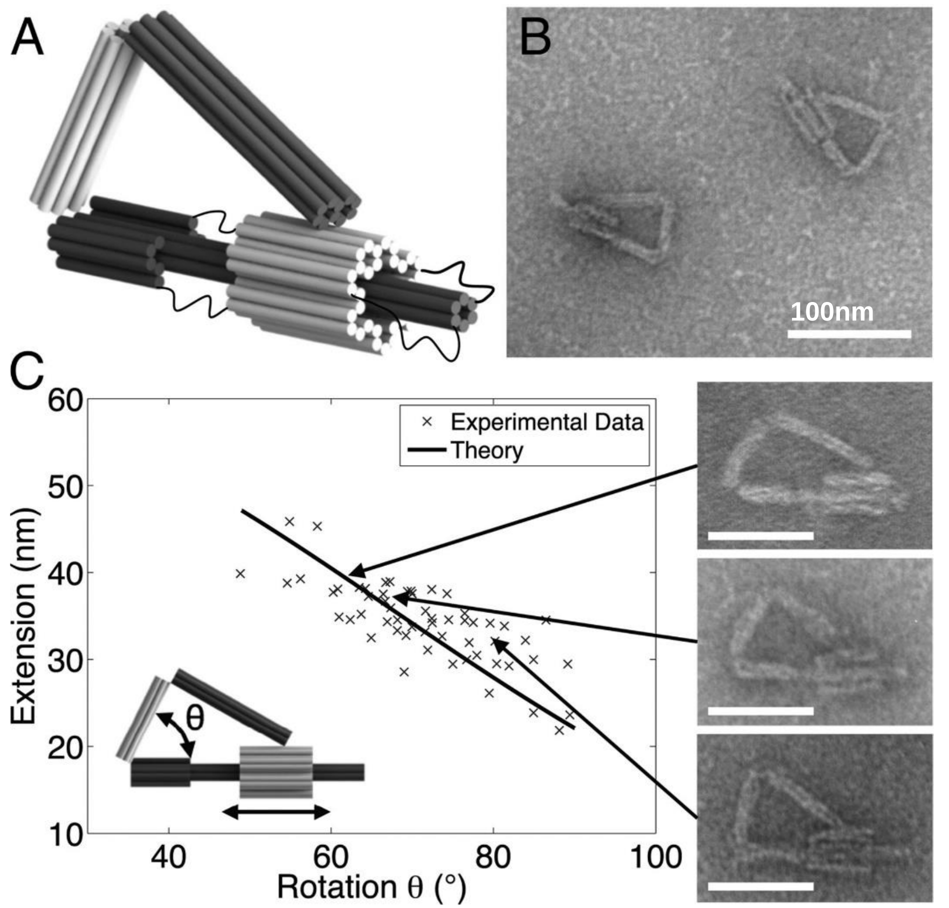



Gap size
three-layer structure
5.2 ± 0.5 nm
one-layer frame
1.7 ± 0.4 nm
Schematic
TEM image
50 nm
50 nm
50 nm
50 nm

Programmable motion of DNA origami mechanisms

Alexander E. Marras, Lifeng Zhou, Hai-Jun Su, and Carlos E. Castro[1]

Proc. Natl Acad. Sci. USA **112**, 713-718 (2015)



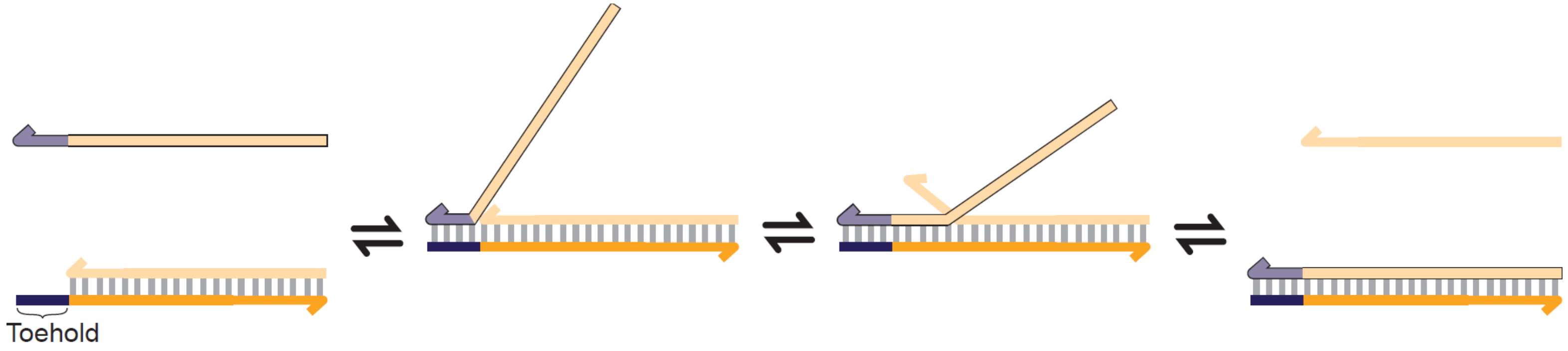
So far –

- sequence-specific hybridization can be used to program the self-assembly of DNA and RNA nanostructures.
- nucleic acid nanostructures can act as atomically precise scaffolds

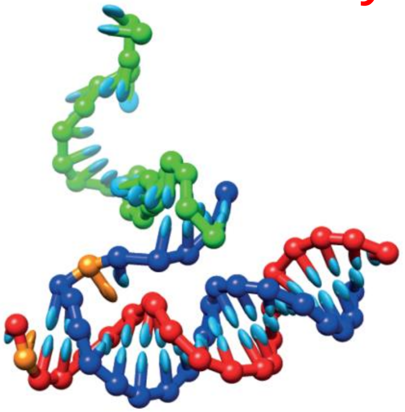
Next –

- dynamics ...

Toehold-mediated strand displacement



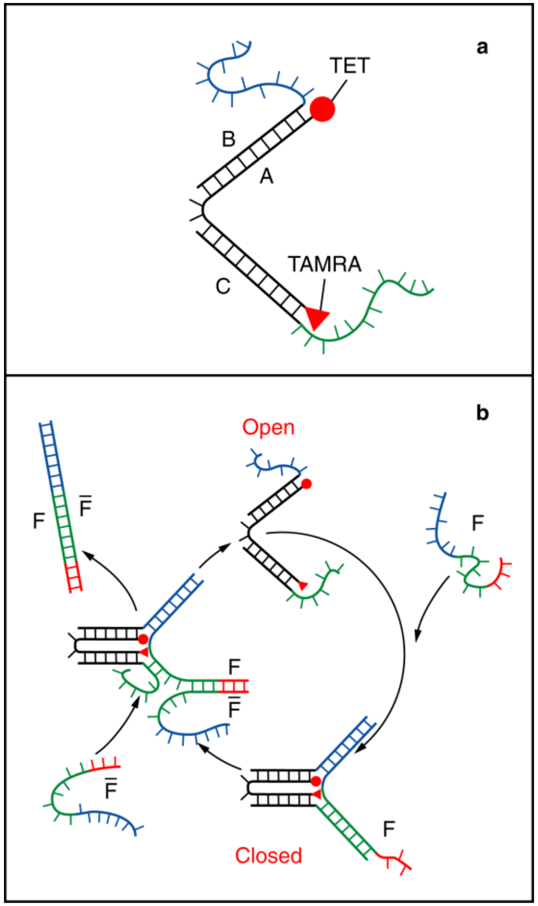
… underpins most current research in dynamic DNA nanotechnology, including synthetic molecular machinery and molecular computation

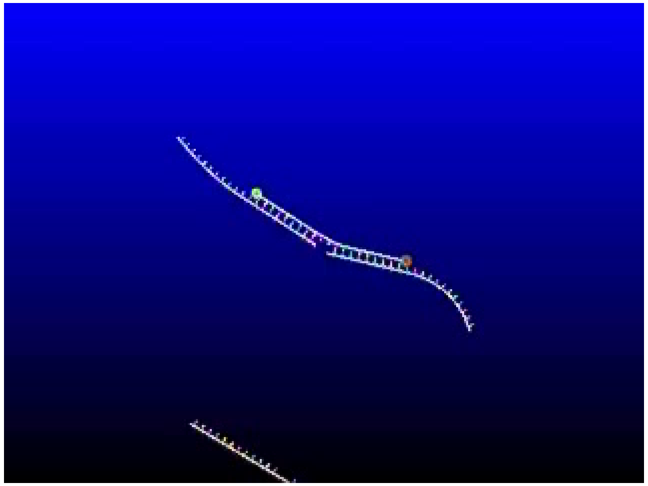


A DNA-fuelled molecular machine made of DNA

Bernard Yurke*, Andrew J. Turberfield*†, Allen P. Mills Jr*, Friedrich C. Simmel* & Jennifer L. Neumann*

Nature **406**, 605-608 (2000)





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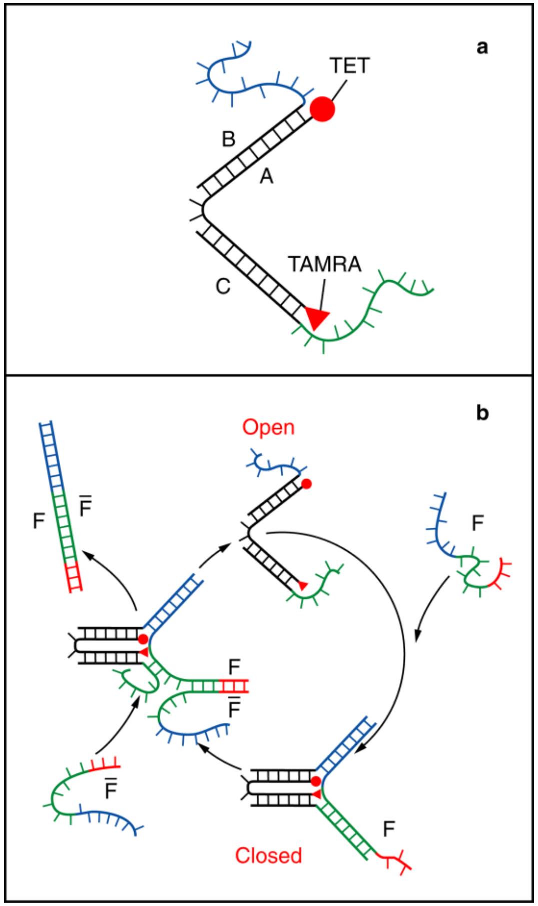
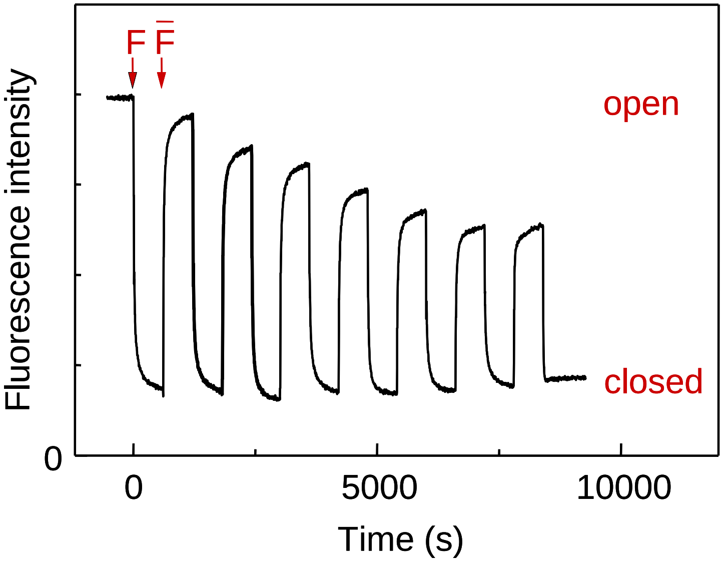
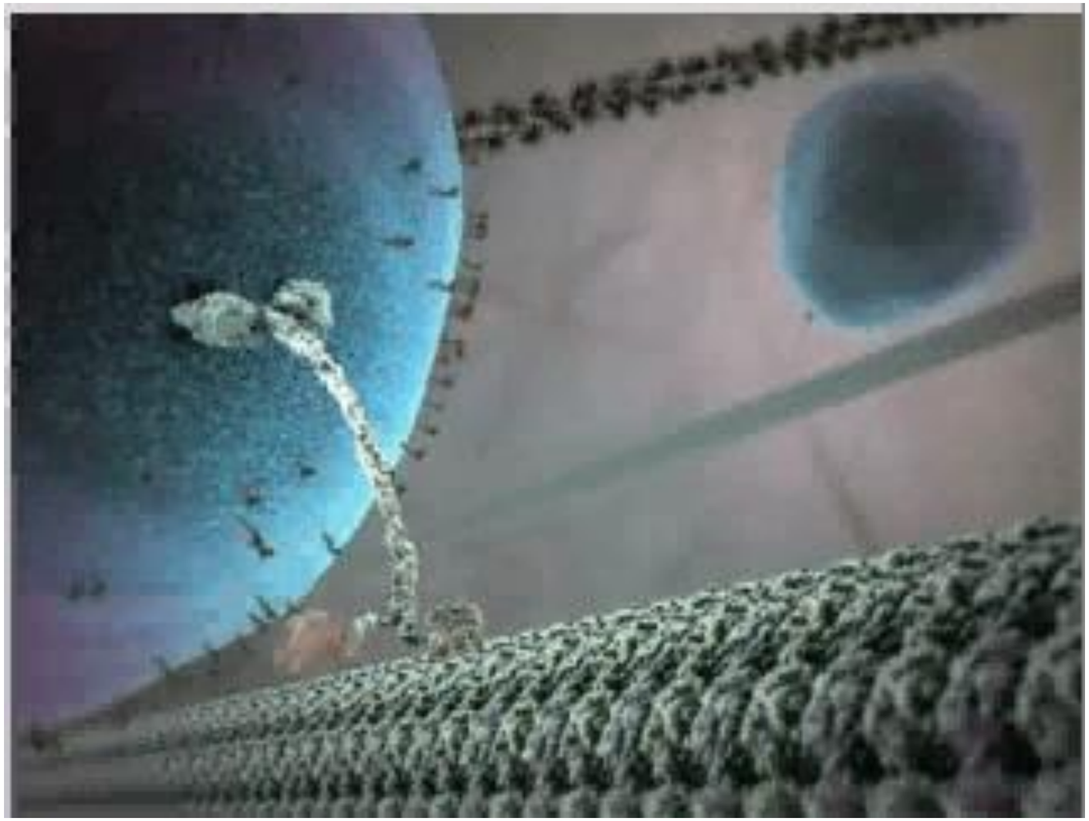




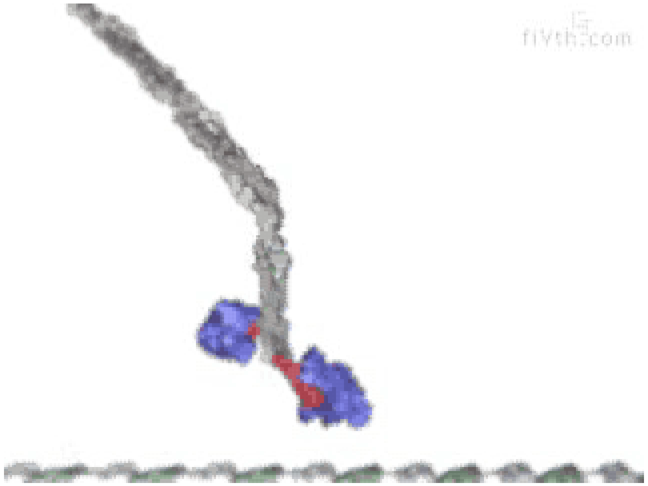


from The Inner Life of the Cell, Harvard

Aim: to build a synthetic molecular motor

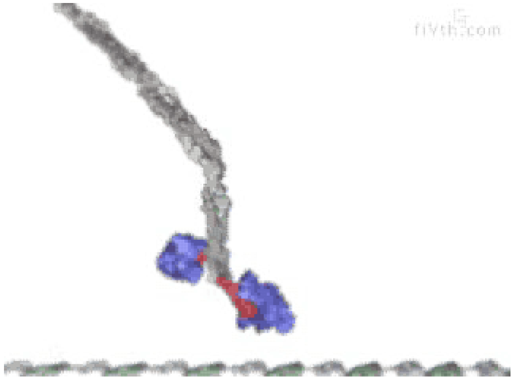
- capable of transporting a load along a track
- chemically fuelled
- autonomous

Inspiration: kinesin



R.D. Vale and R.A. Milligan, *Science* **288**, 88 (2000)

- transport along microtubules: proteins, mRNA, organelles, DNA (cell division)
- powered by ATP: motor catalyzes hydrolysis $\Delta G \approx -12$ kcal mol^{-1}
- fast (1 µm s^{-1} in 8 nm steps)
- processive (hundreds of steps without falling off)
- essential that catalytic activities of the two identical feet are coordinated



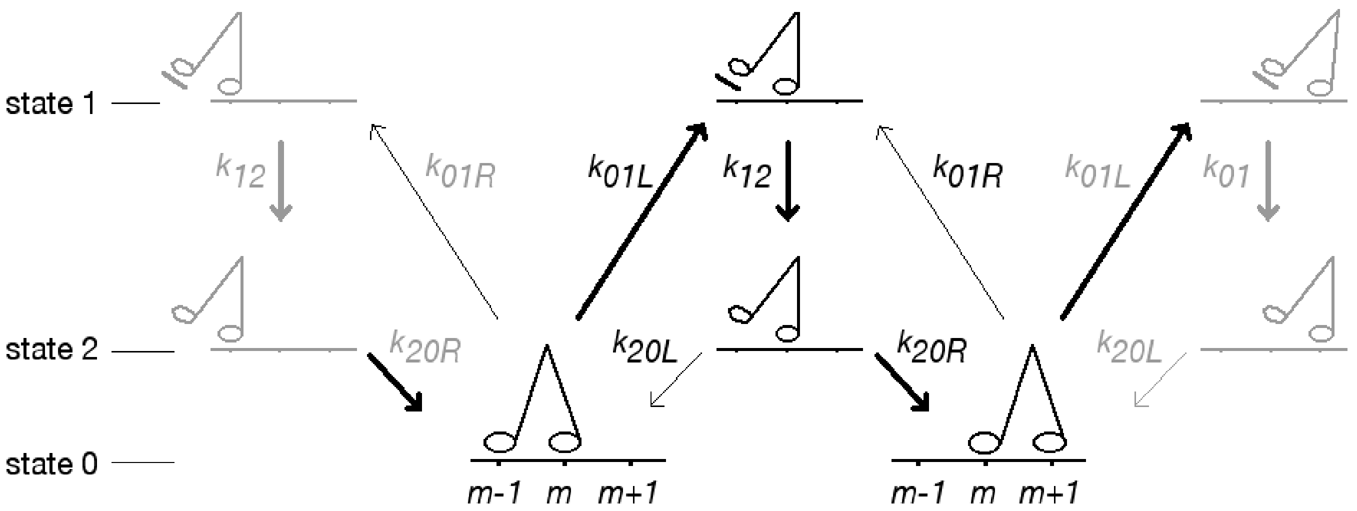
What do we need to make an autonomous, chemically powered motor?

- fuel
- motor catalyzes reaction of fuel
- reaction of fuel coupled to mechanical motion

and for a two-footed motor:

- coordinate chemomechanical cycles of feet
  to achieve directionality, processivity

Directionality through control of transition rates



$$\alpha = \frac{k_{01L}}{k_{01R}}$$

$$\beta = \frac{k_{20L}}{k_{20R}}$$

0→1 fuel lifts foot from track

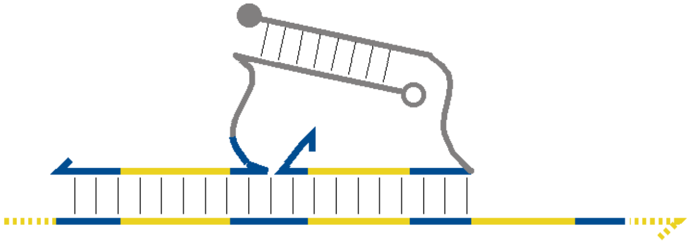
1→2 foot catalyzes reaction of fuel, fuel displaced

2→3 foot rebinds track

- each transition is thermodynamically downhill
- dissipation of free energy by reaction of fuel uncouples foot lifting and replacing

→ **possibility of directional bias by control of reaction rates** $\frac{\alpha}{\beta} \neq 1$

Coordinating two chemomechanical cycles

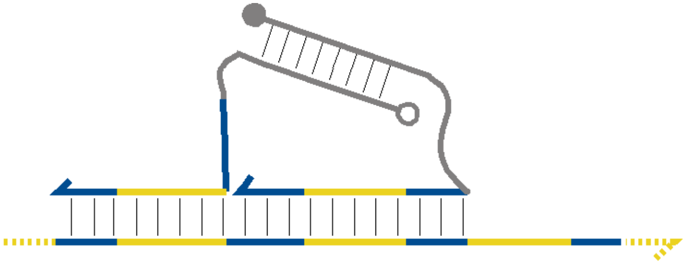


Mechanism for coordinated motion of a synthetic molecular motor

S.J. Green, J. Bath, A.J. Turberfield *Phys. Rev. Lett.* **101**, 238101 (2008)

J. Bath, S.J. Green, K.E. Allen, A.J. Turberfield *Small* ***5**, 1513-1516 (2009)*

Coordinating two chemomechanical cycles



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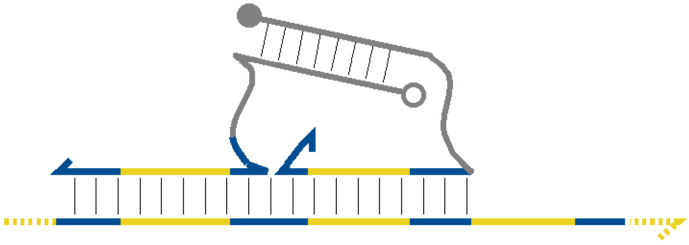

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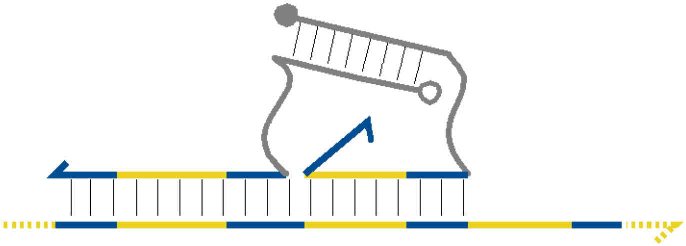

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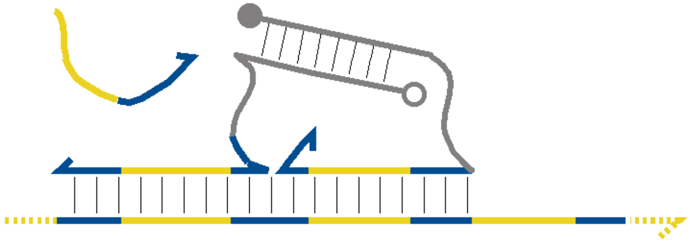

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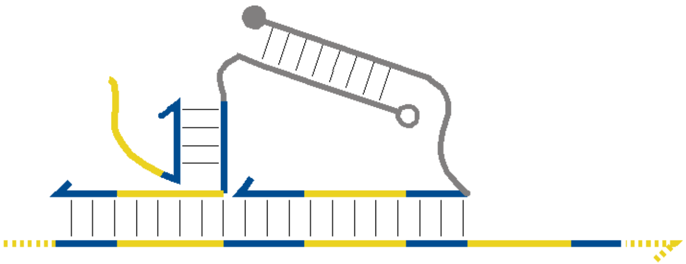
Coordinating two chemomechanical cycles



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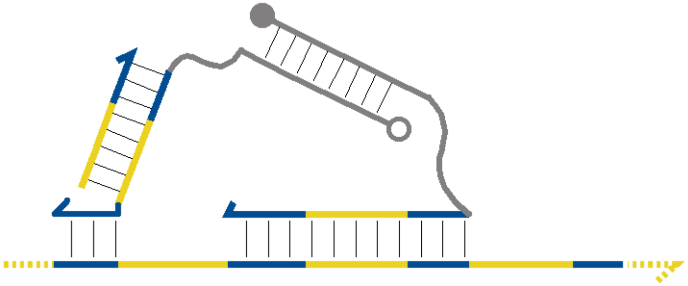

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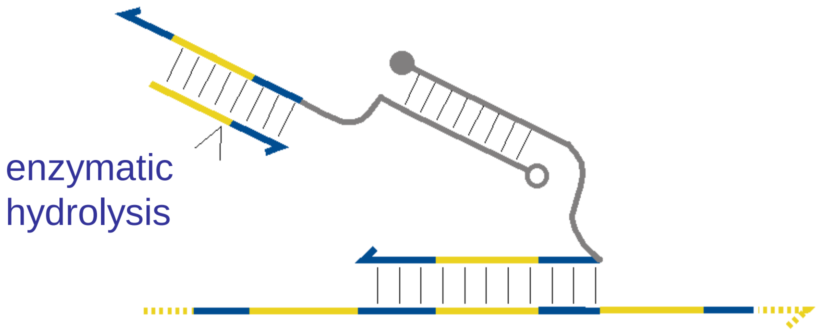
Coordinating two chemomechanical cycles



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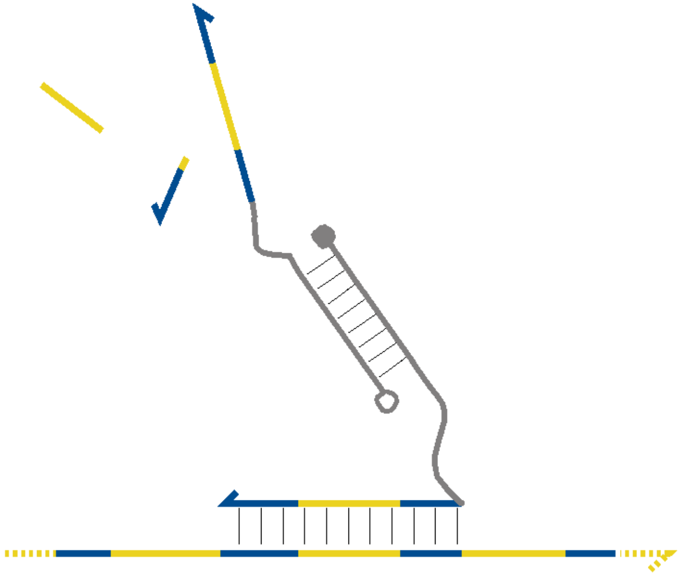

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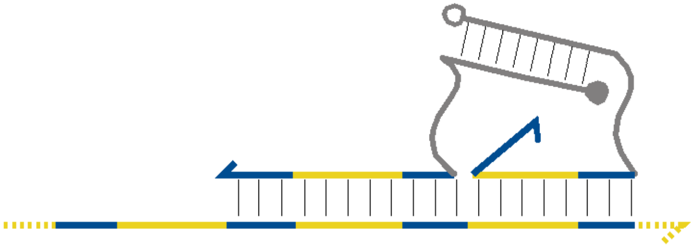

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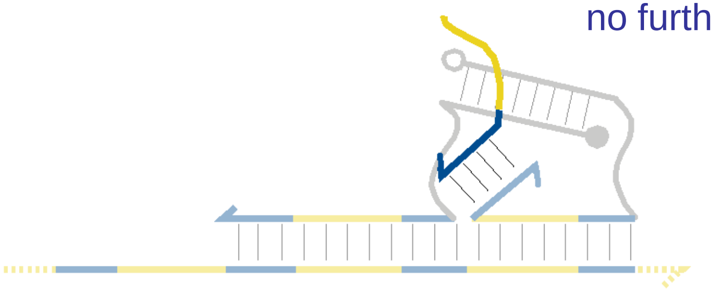


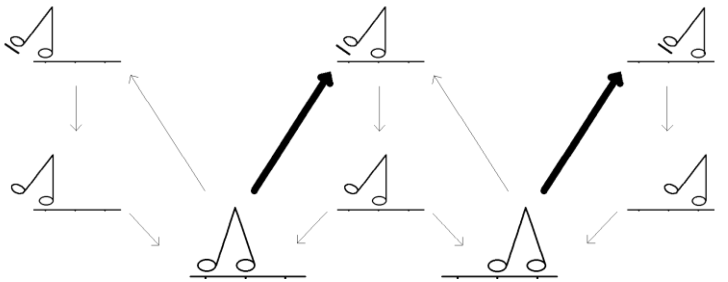
fuel binds to wrong foot –
no further reaction

Mechanism for coordinated motion of a synthetic molecular motor

S.J. Green, J. Bath, A.J. Turberfield *Phys. Rev. Lett.* **101**, 238101 (2008)

J. Bath, S.J. Green, K.E. Allen, A.J. Turberfield *Small **5**, 1513-1516 (2009)*

the mechanism is both directional and processive



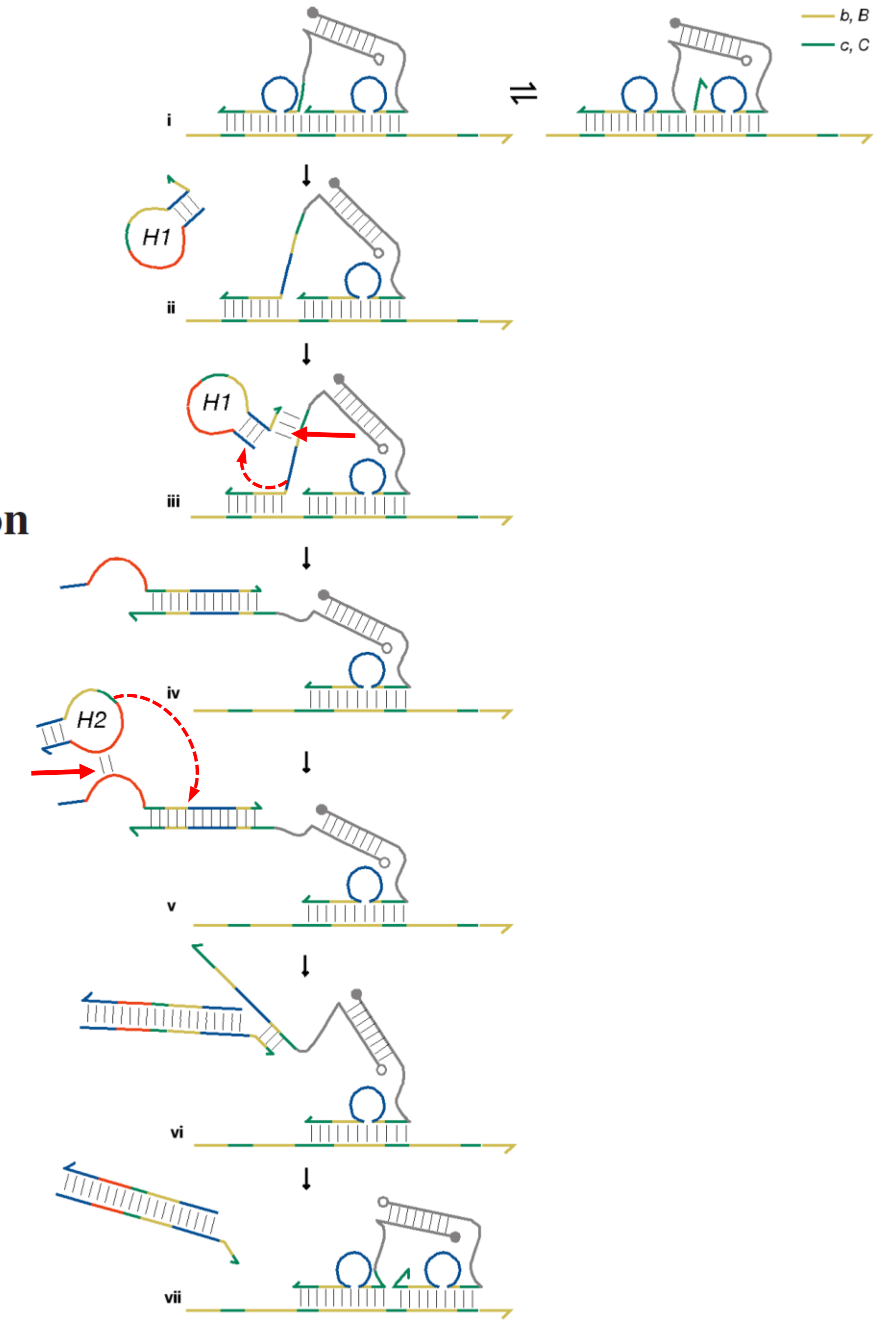
- engineered 30-fold bias toward lifting the left foot
- once the left foot is lifted the standing foot is unlikely to be lifted from the track
- there is no intrinsic bias in replacing a lifted foot either side of the standing foot

→ **the mechanism works as designed**

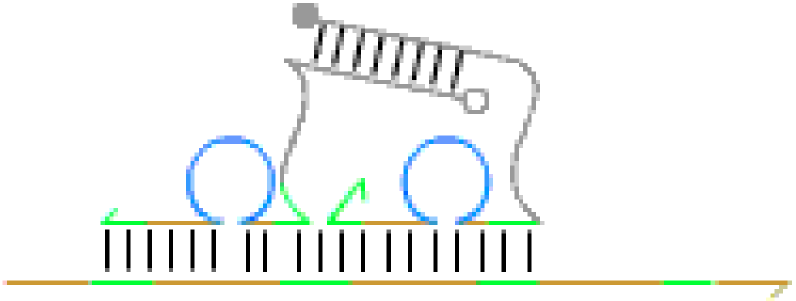
Coordinated Chemomechanical Cycles: A Mechanism for Autonomous Molecular Motion

S. J. Green, J. Bath, and A. J. Turberfield

Phys. Rev. Lett. **101**, 238101 (2008)



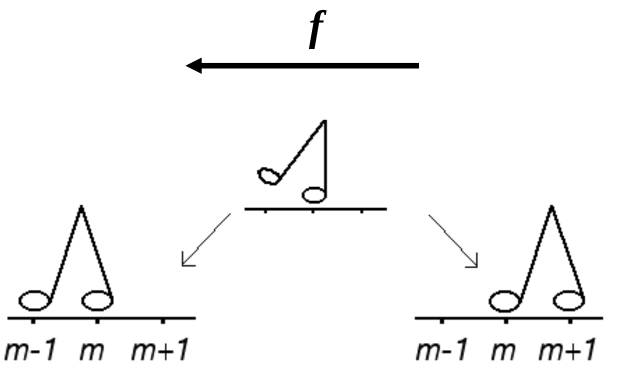
Coordinated chemomechanical cycles: a mechanism for autonomous molecular motion



S.J. Green, J. Bath and A.J. Turberfield
Phys. Rev. Lett. **101**, 238101 (2008)

These motors are **Brownian ratchets**: movement of the lifted foot is driven solely by thermal fluctuations; the fuel provides the energy necessary to rectify this motion by breaking the detailed balance between lifting and replacing front and back feet.

Model load *f* by favouring foot replacement in left (back) position by a factor $\exp(fx/k_BT)$ where x is the distance between transition states for forward and backward steps (set $x \approx$ step size d).



velocity $$v = k_{\text{eff}} d \left(\frac{1}{1+\alpha^{-1}} - \frac{1}{1+\beta^{-1} e^{-fd/k_BT}} \right),$$

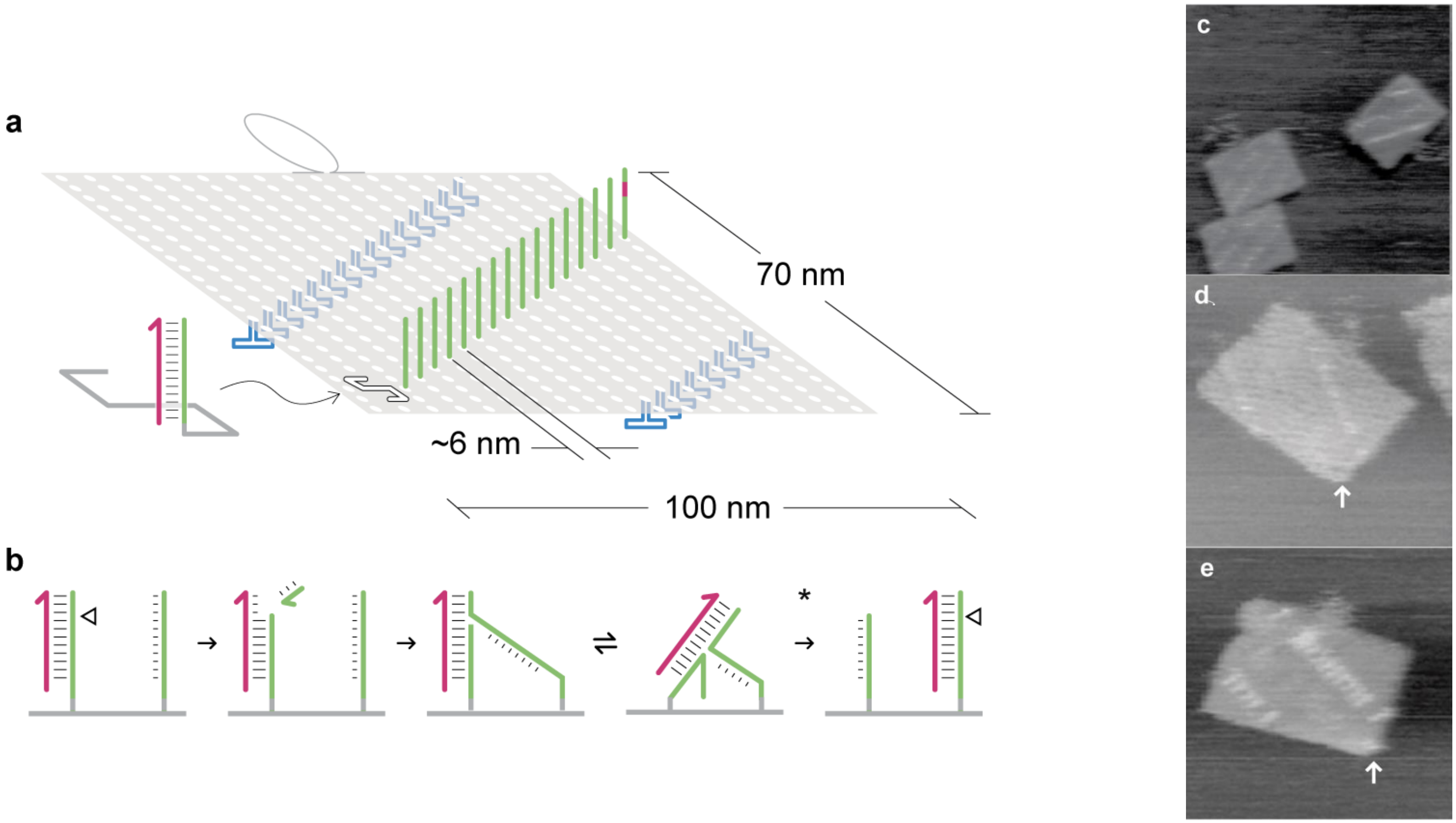
where

$$\begin{aligned} k_{\text{eff}}^{-1} &= (k_{01L} + k_{01R})^{-1} + k_{12}^{-1} \\ &+ \left(k_{20L} e^{fd/2k_BT} + k_{20R} e^{-fd/2k_BT} \right)^{-1}. \end{aligned}$$

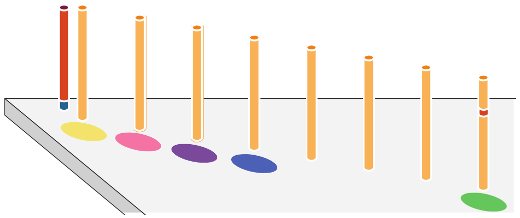
set $v = 0$:

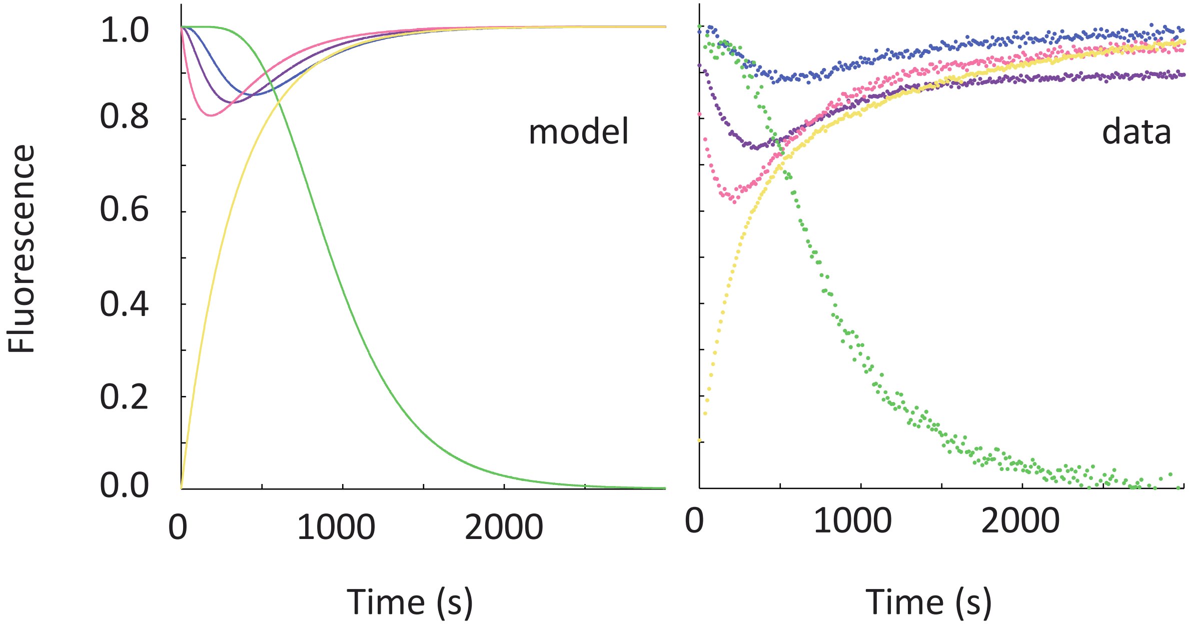
$$f_{stall} = \frac{k_BT}{d} ln \frac{\alpha}{\beta} \approx 3 \text{ pN}$$

DNA walker mechanism



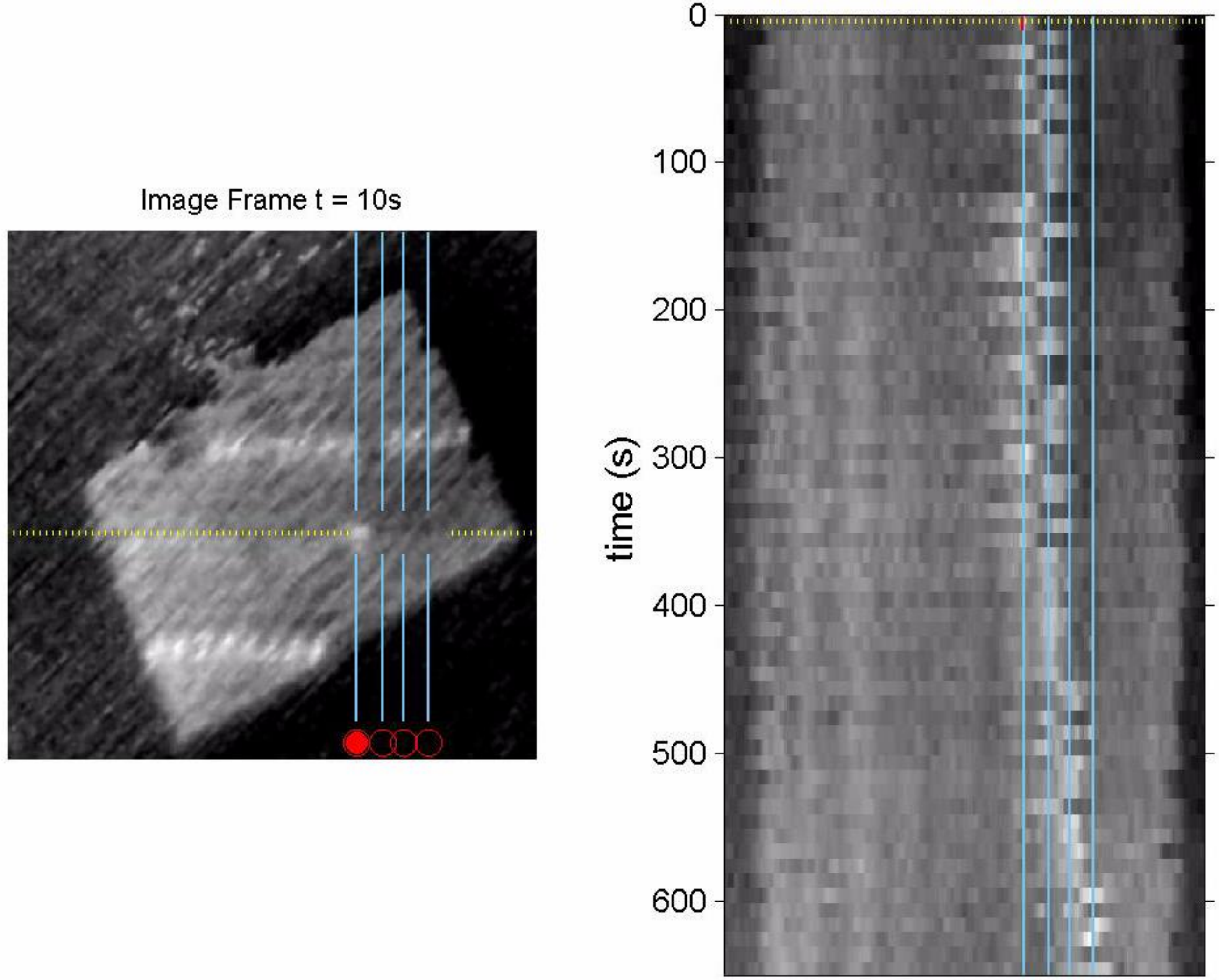
Direct observation of stepwise movement of a synthetic molecular transporter
S. F. J. Wickham, M. Endo, Y. Katsuda, K. Hidaka, J. Bath, H. Sugiyama and A. J. Turberfield
Nature Nanotechnol. **6**, 166 (2011)





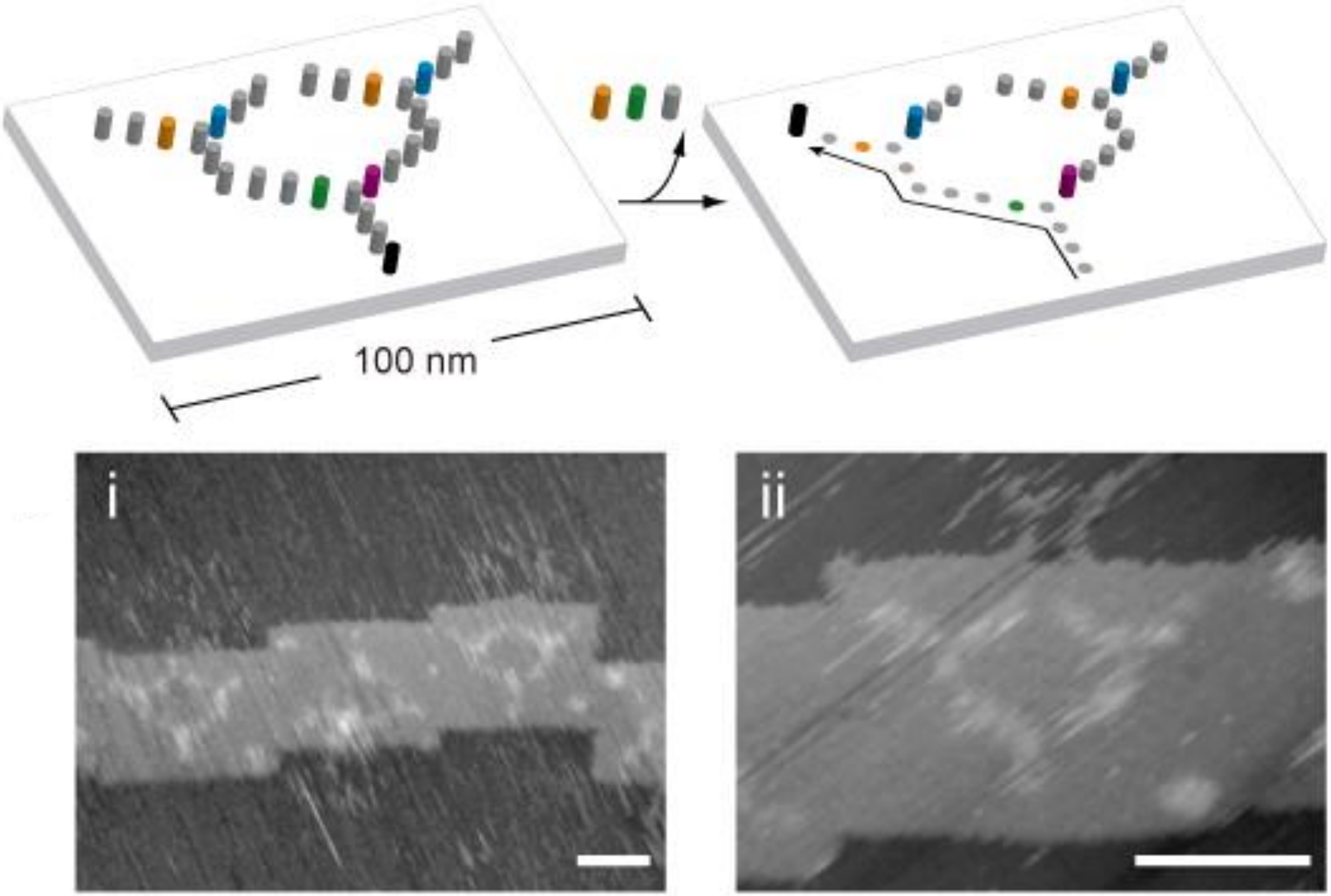
Direct observation of stepwise movement of a synthetic molecular transporter
S. F. J. Wickham, M. Endo, Y. Katsuda, K. Hidaka, J. Bath, H. Sugiyama and A. J. Turberfield
Nature Nanotechnol. **6**, 166 (2011)

Individual steps resolved by real-time AFM



Direct observation of stepwise movement of a synthetic molecular transporter
S. F. J. Wickham, M. Endo, Y. Katsuda, K. Hidaka, J. Bath, H. Sugiyama and A. J. Turberfield
Nature Nanotechnol. **6**, 166 (2011)

Navigating a network of tracks

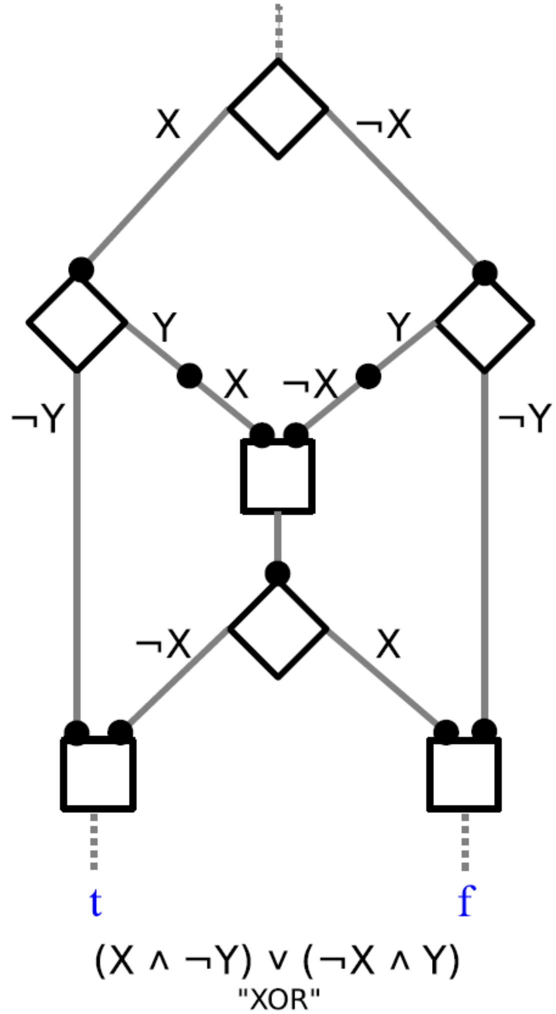


Molecules that navigate a network of tracks
S. F. J. Wickham, J. Bath, Y. Katsuda, M. Endo, K. Hidaka, H. Sugiyama and A. J. Turberfield,
Nature Nanotechnol. **6**, 166 (2011)

Localized computing by selectively blocking DNA walkers

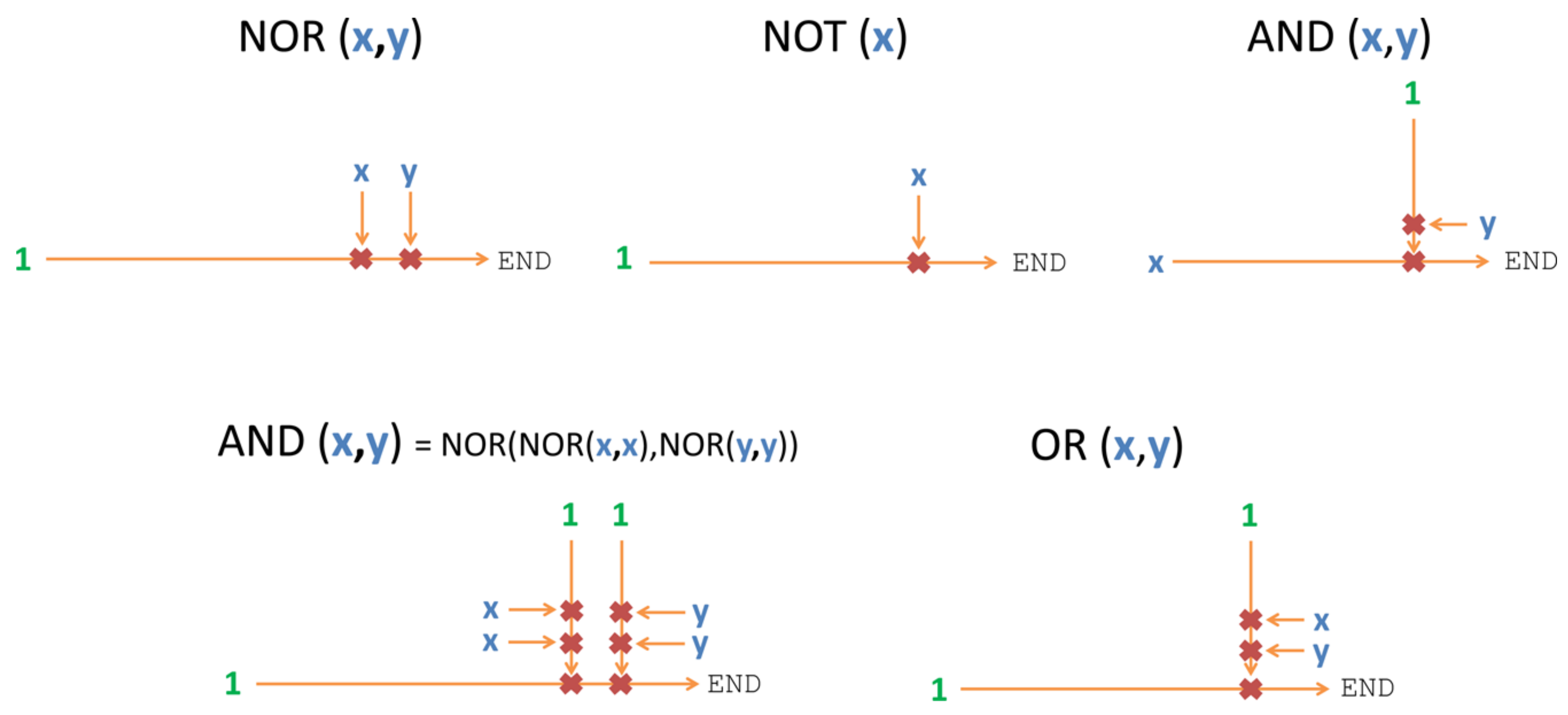
Composable

XOR circuit



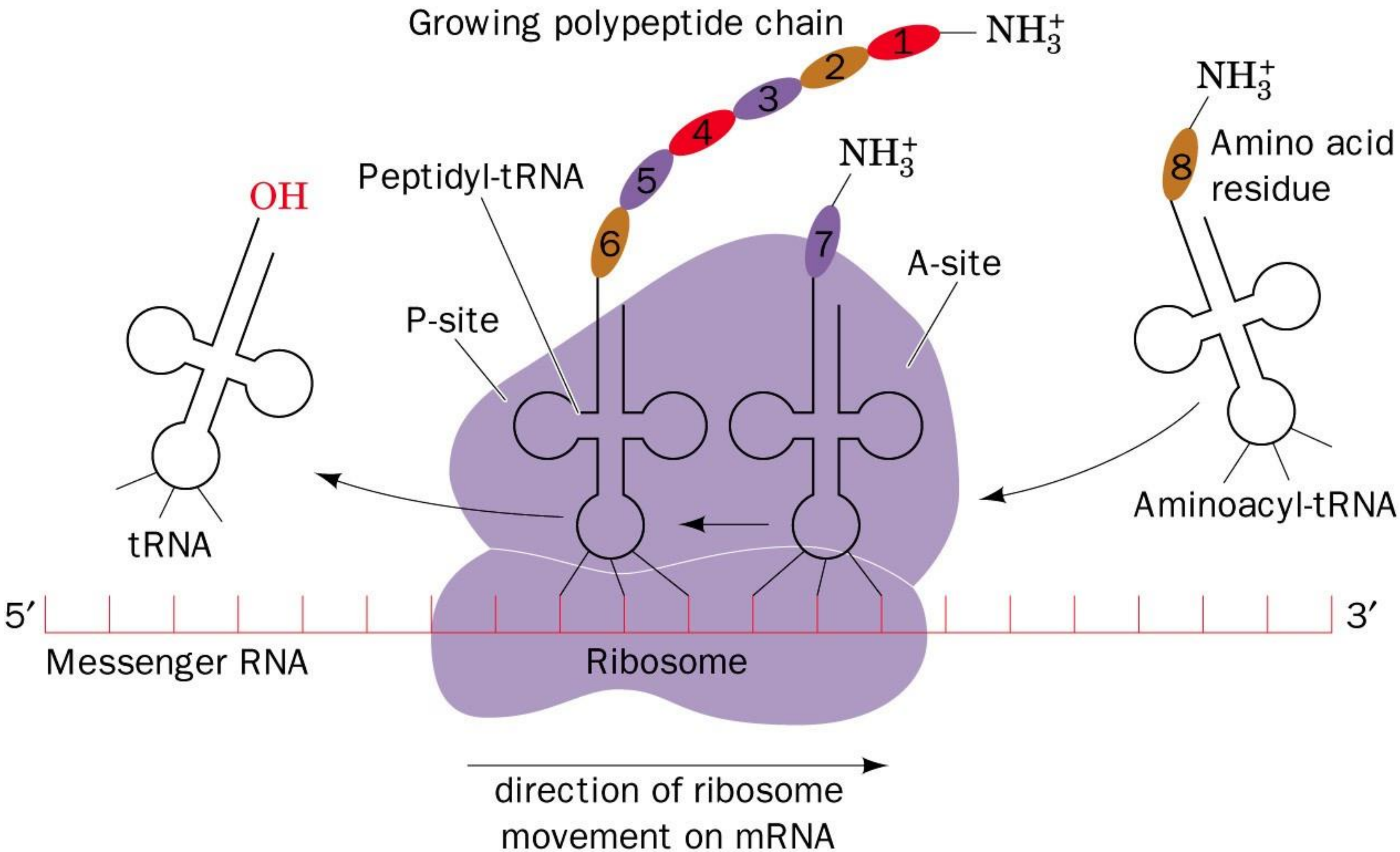
DNA walker circuits: computational potential, design and verification
F. Dannenberg, M. Kwiatkowska, C. Thachuk and A. J. Turberfield
Nat. Comput. **14**, 195-211 (2015)

Computing with interacting DNA walkers



The Formal Language and Design Principles of Autonomous DNA Walker Circuits
M. A. Boemo, A. E. Lucas, A. J. Turberfield and L. Cardelli
ACS Synth. Biol. **5**, 878-884 (2016)

DNA-templated chemistry – towards a synthetic ribosome



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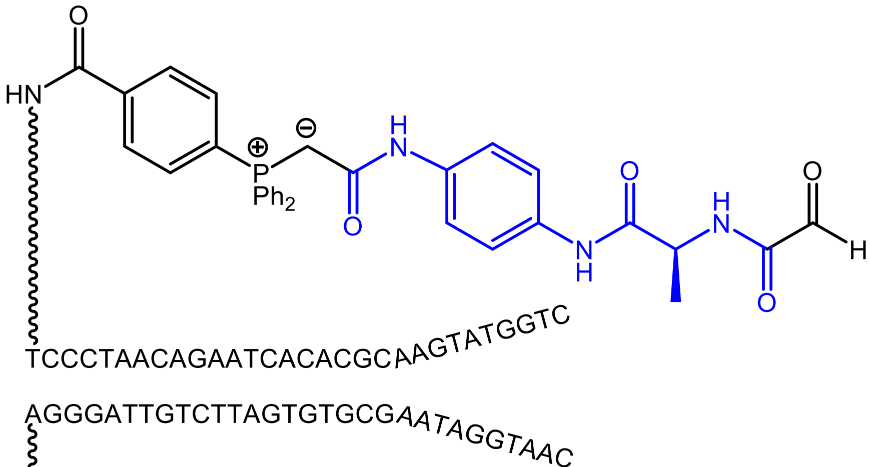
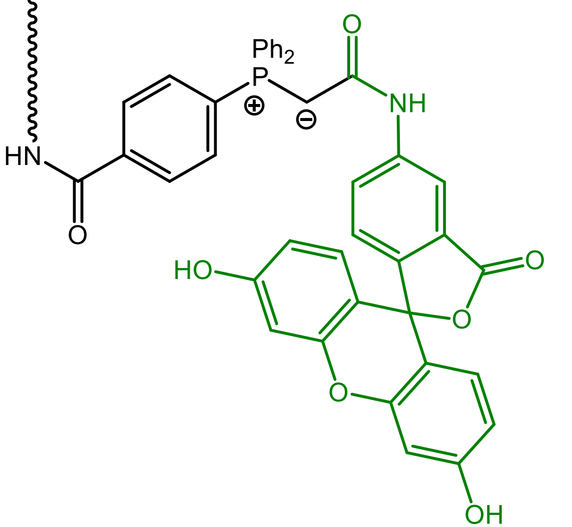
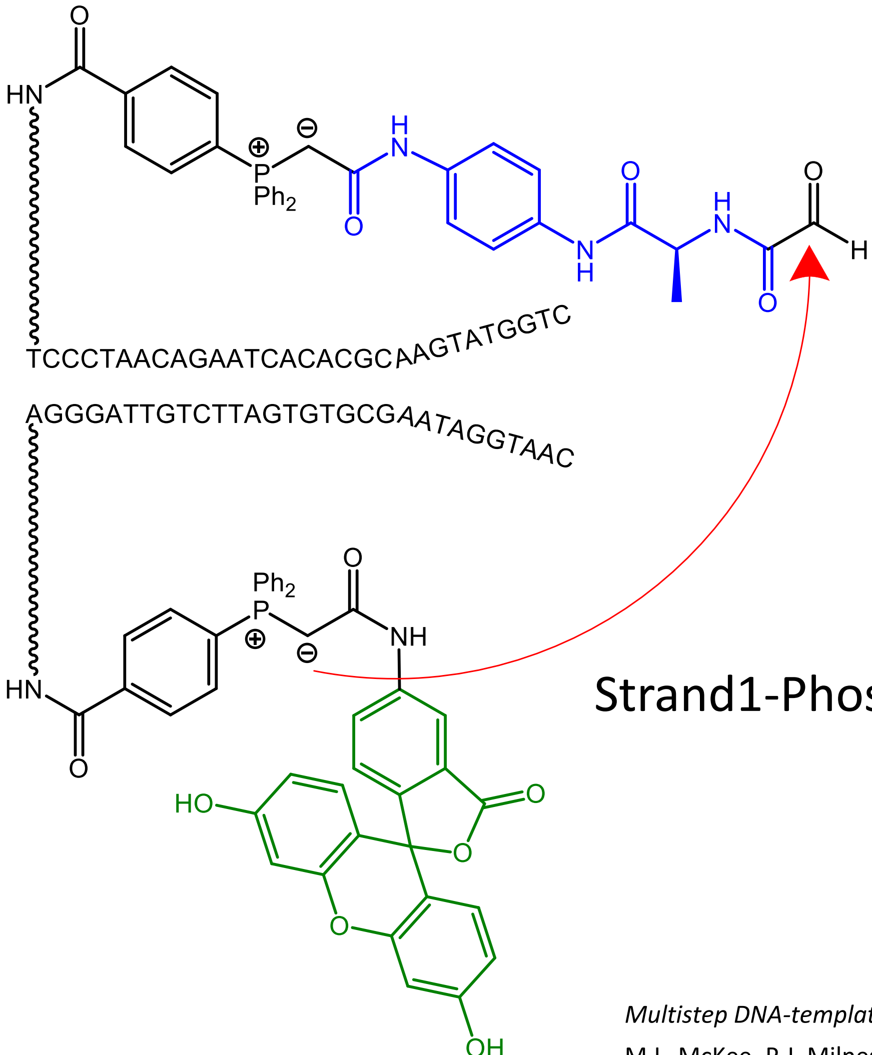


Strand2-Phos-Ala-Ald



Strand1-Phos-FAM

Multistep DNA-templated reactions for the synthesis of functional sequence controlled oligomers.
M.L. McKee, P.J. Milnes, J. Bath, E. Stulz, A.J. Turberfield, R.K. O'Reilly
Angew. Chem. Int. Ed. **49**, 7948 (2010)



Strand2-Phos-Ala-Ald

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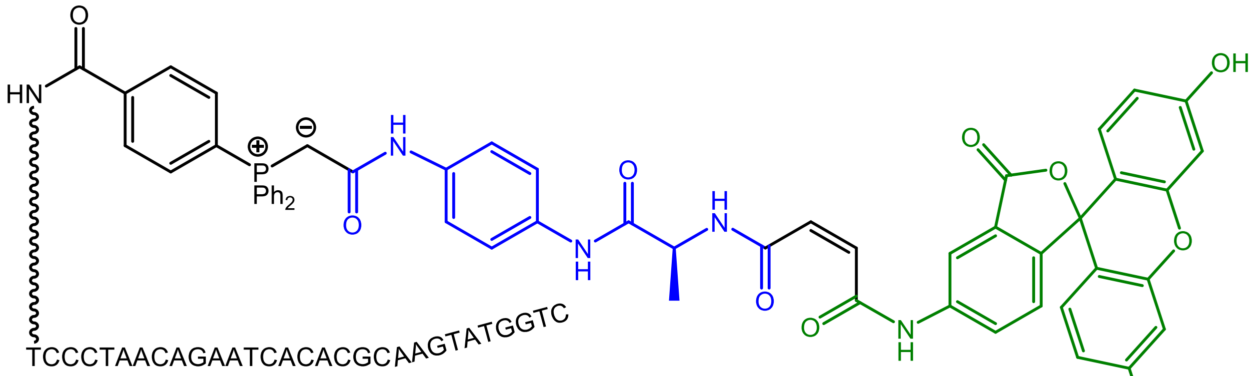
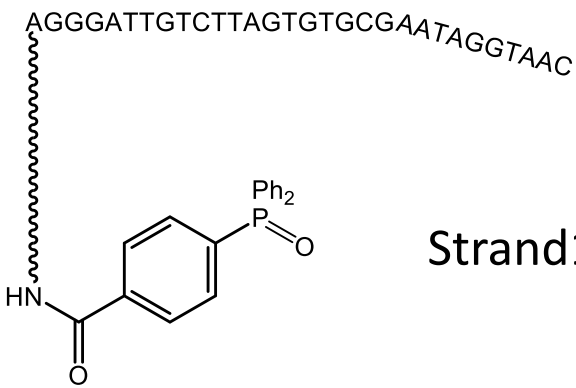


Strand2-Phos-Ala-FAM



Strand1-Phos-Oxide

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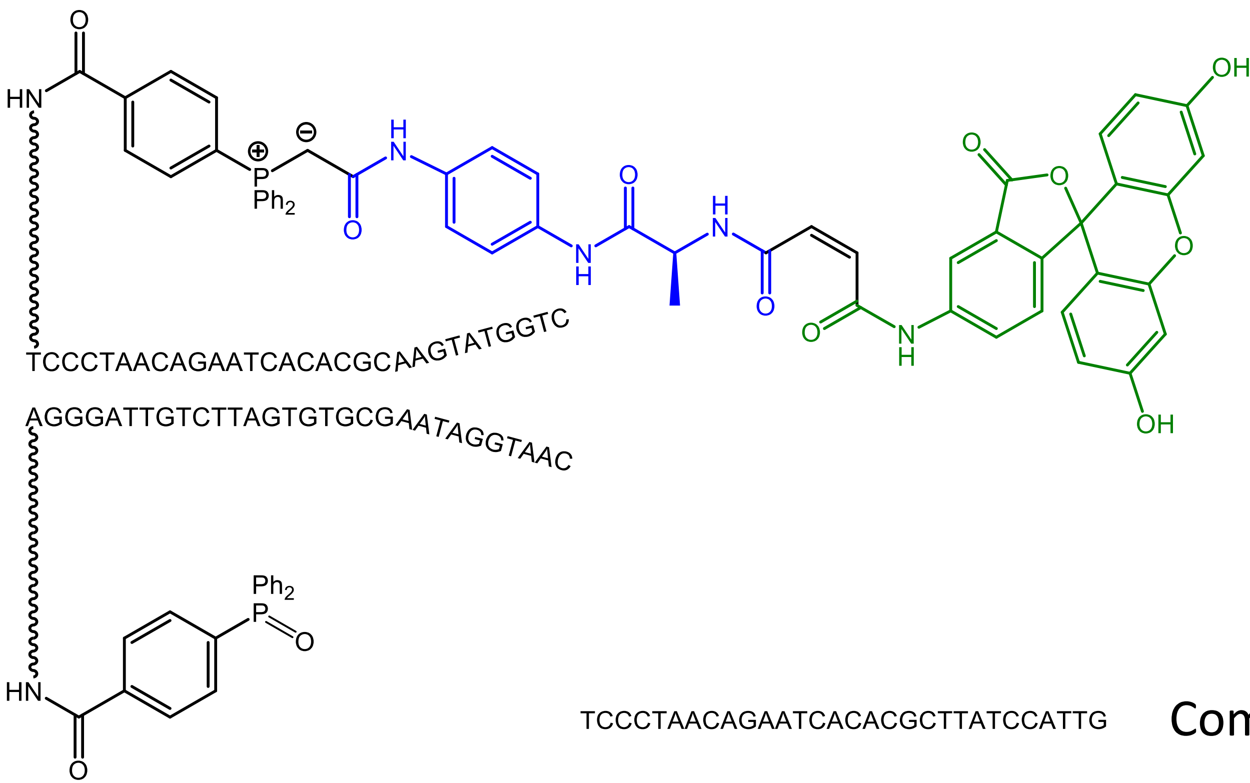


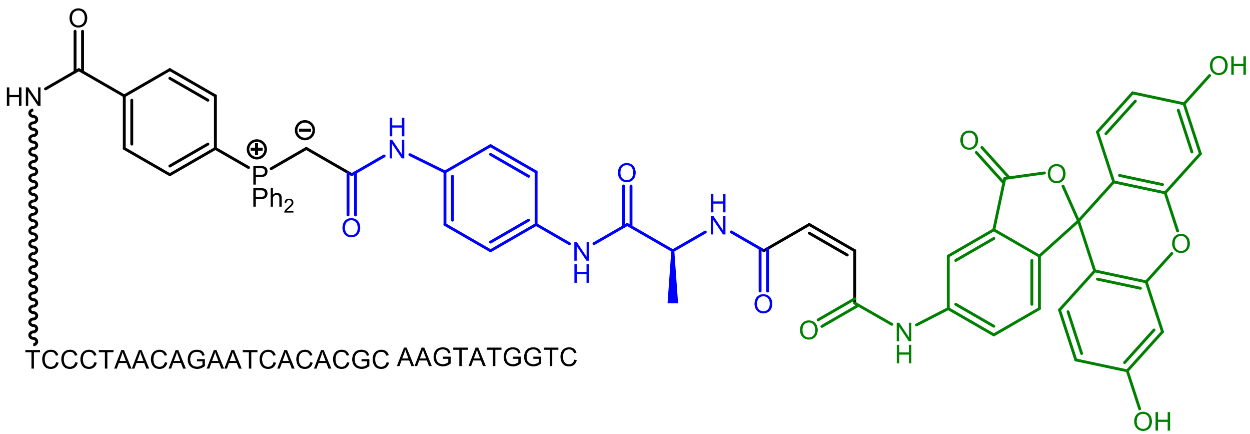
Strand2-Phos-Ala-FAM

Complementary1

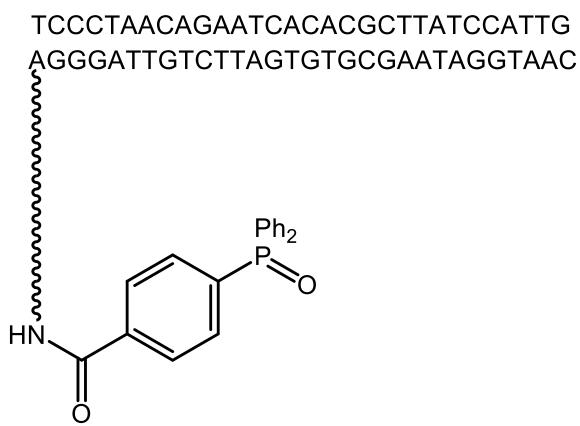
Multistep DNA-templated reactions for the synthesis of functional sequence controlled oligomers.

M.L. McKee, P.J. Milnes, J. Bath, E. Stulz, A.J. Turberfield, R.K. O'Reilly

Angew. Chem. Int. Ed. **49**, 7948 (2010)



Strand2-Phos-Ala-FAM



Complementary1
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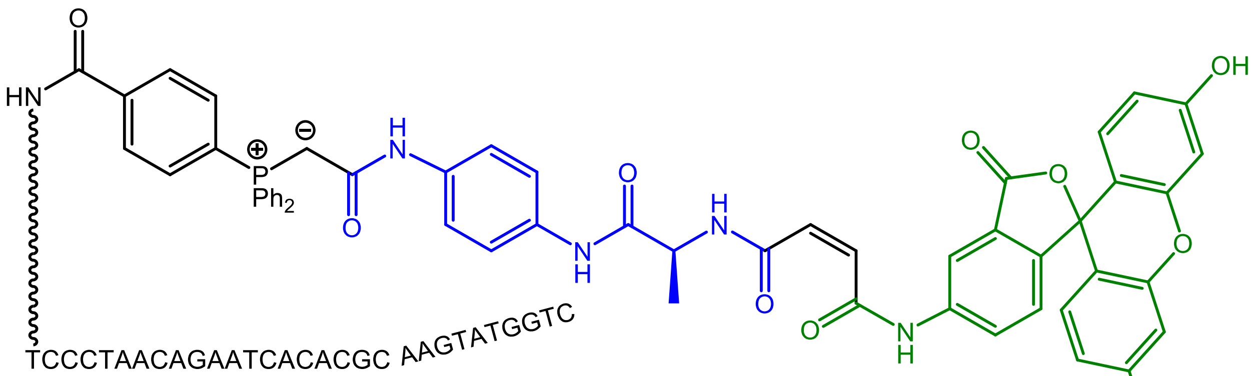
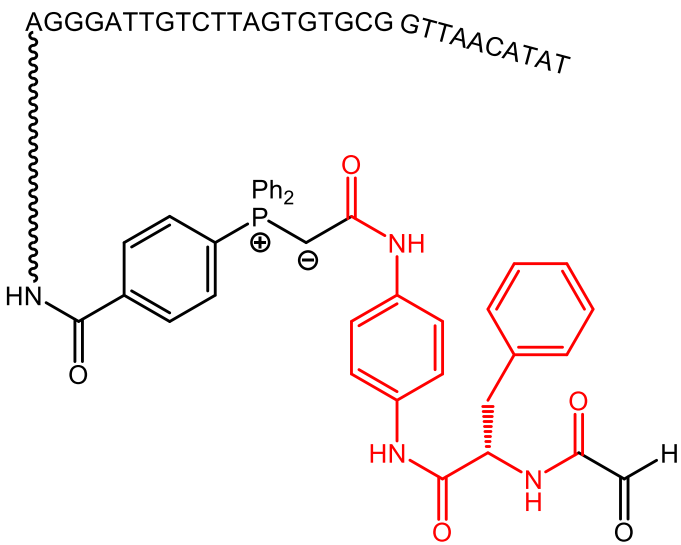


Strand2-Phos-Ala-FAM



Strand3-Phos-Phe-Ald

Multistep DNA-templated reactions for the synthesis of functional sequence controlled oligomers.
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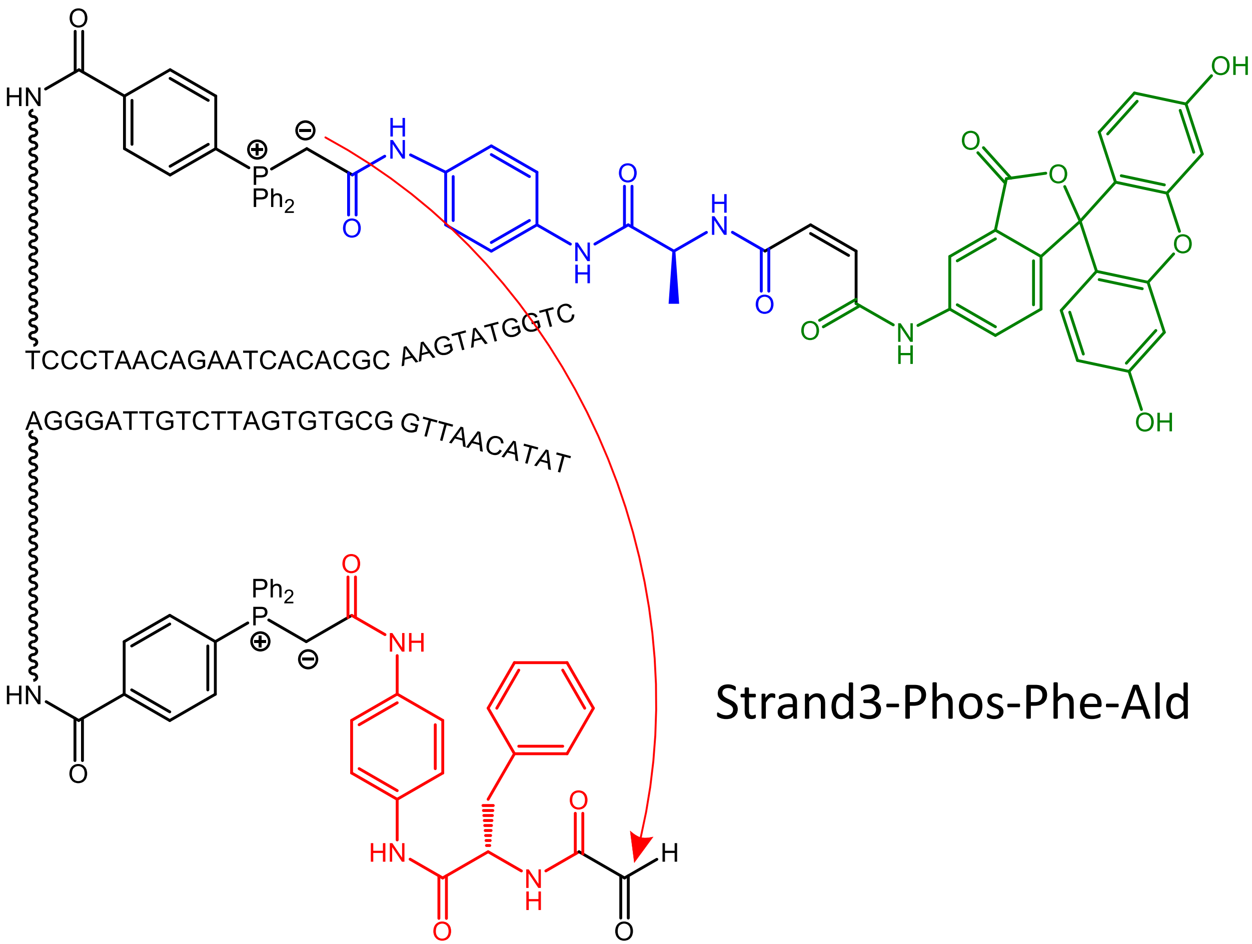

Strand2-Phos-Ala-FAM

Strand3-Phos-Phe-Ald

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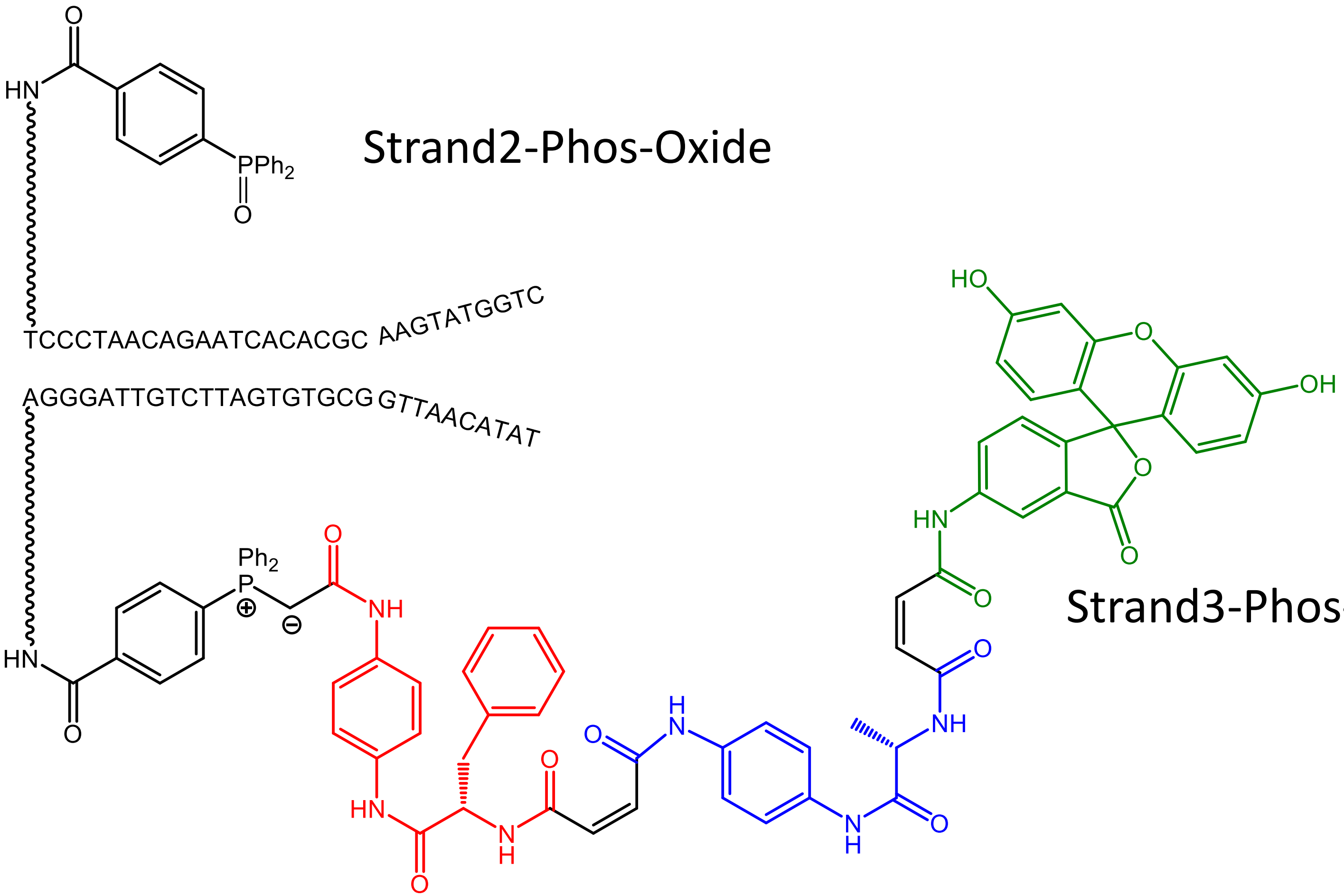

Strand2-Phos-Oxide

Strand3-Phos-Phe-Ala-FAM

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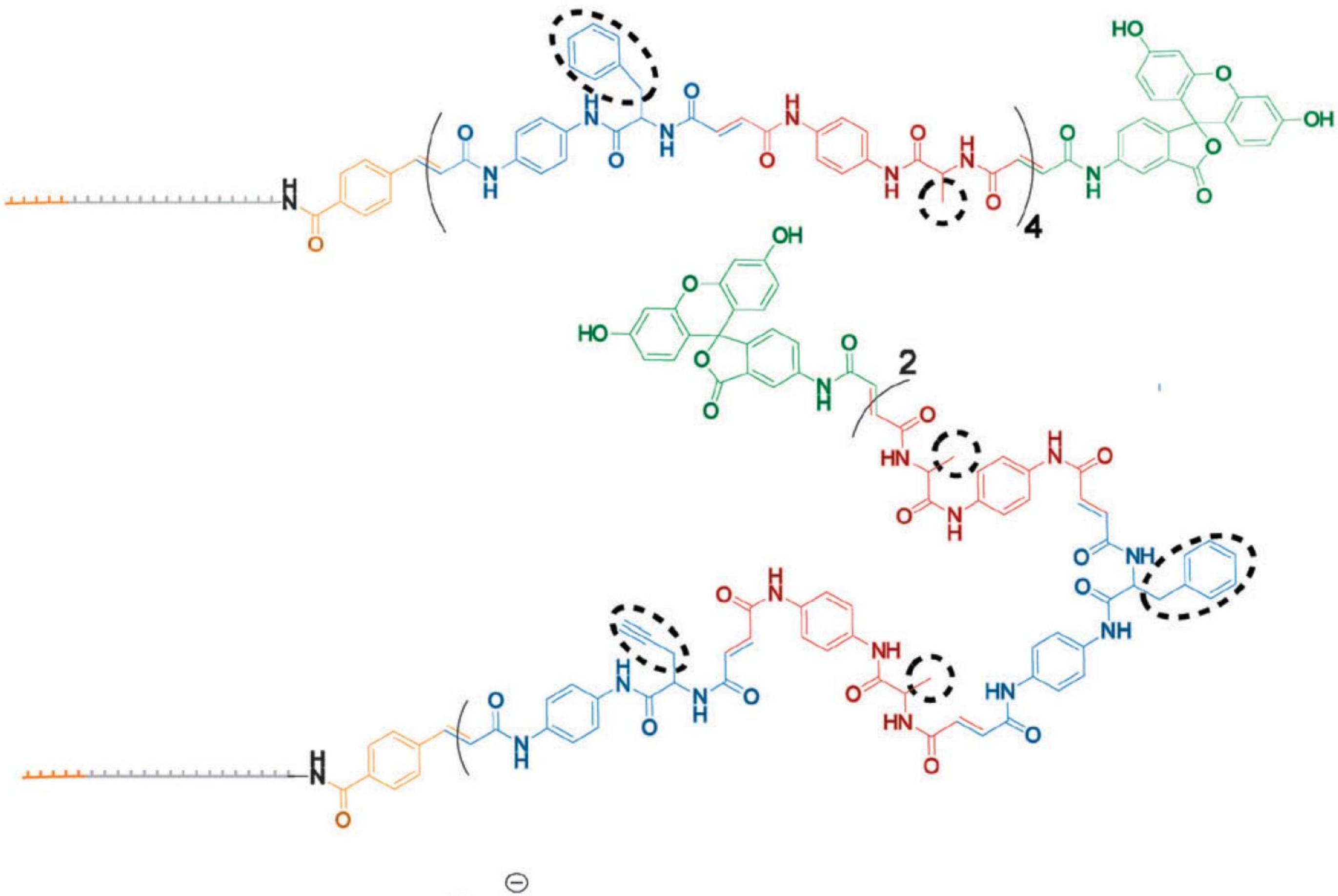
Angew. Chem. Int. Ed. **49**, 7948 (2010)

DNA-templated chemistry – 10-mers!



88% yield per step

85% yield per step

Sequence-specific synthesis of macromolecules using DNA-templated chemistry.
P.J. Milnes, M.L. McKee, J. Bath, L. Song, E. Stulz, A.J. Turberfield, R. K. O'Reilly
Chem. Commun. **48**, 5614 (2012)

An Autonomous Molecular Assembler for Programmable Chemical Synthesis

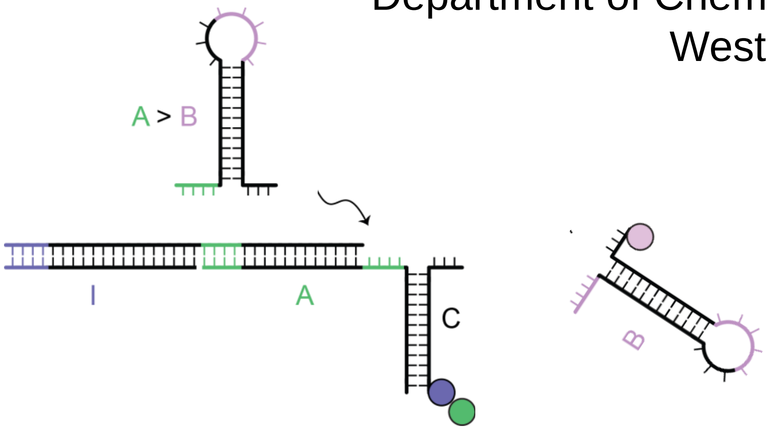
Nature Chem. **8**, 542-8 (2016)

Wenjing Meng, Richard Muscat, Mireya McKee,
Jonathan Bath, Andrew Turberfield

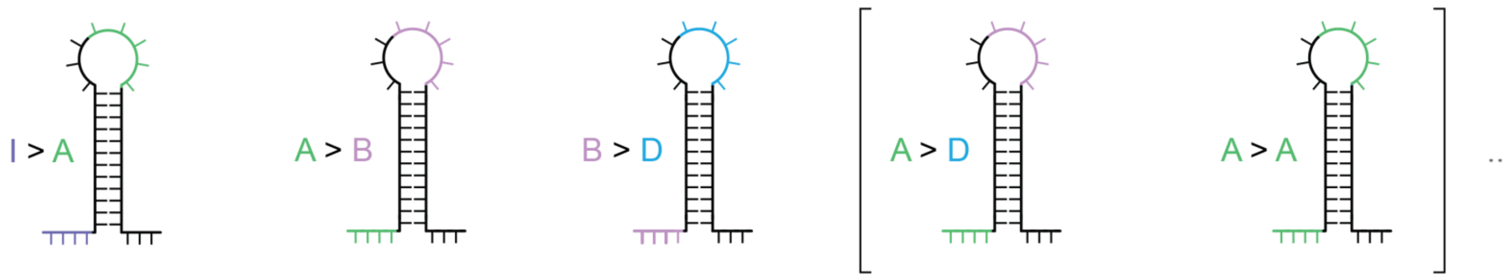
Department of Physics, University of Oxford, Clarendon Laboratory,
Parks Road, Oxford, OX1 3PU, UK

Phillip Milnes, Rachel O'Reilly

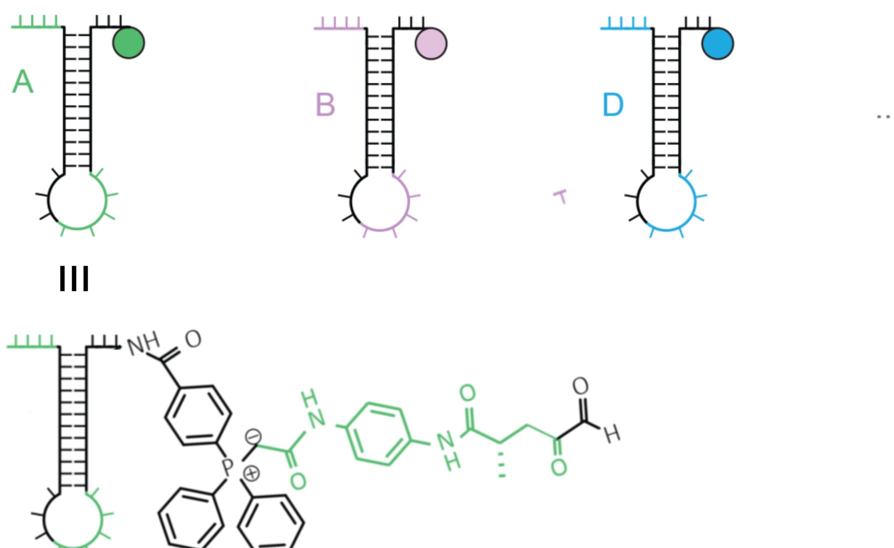
Department of Chemistry, University of Warwick, Coventry,
West Midlands CV4 7AL, UK



Instruction hairpins

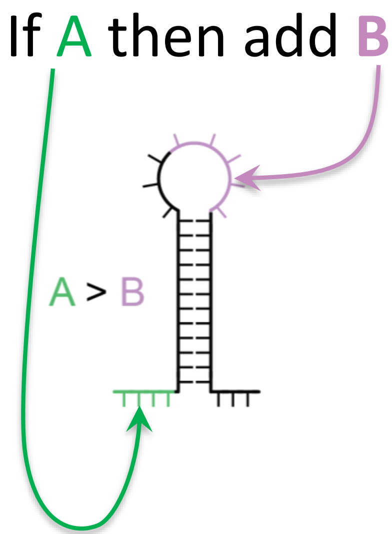


Chemistry hairpins

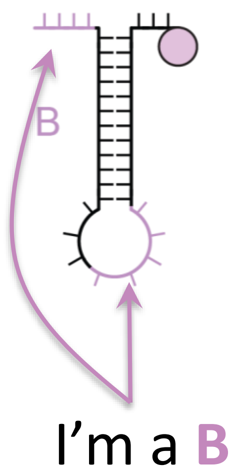


An Autonomous Molecular Assembler for Programmable Chemical Synthesis.
R.A. Muscat, M.L. McKee, P.J. Milnes, J. Bath, R.K. O'Reilly[3] A.J. Turberfield
Nature Chem. **8**, 542-548 (2016)

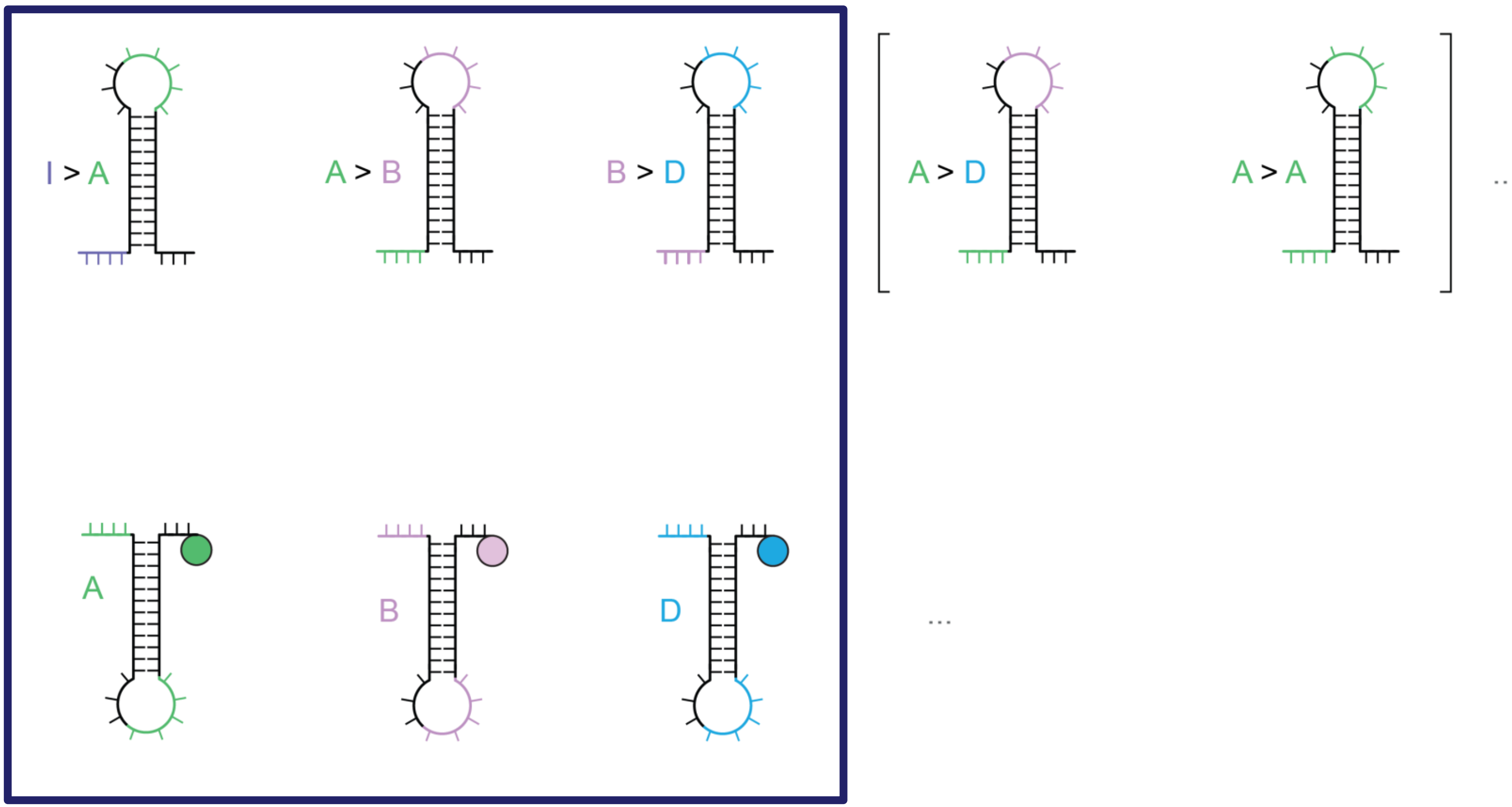
Instruction strand



Chemistry strand



Simple program



Make polymer **A B D** (● ● ●)

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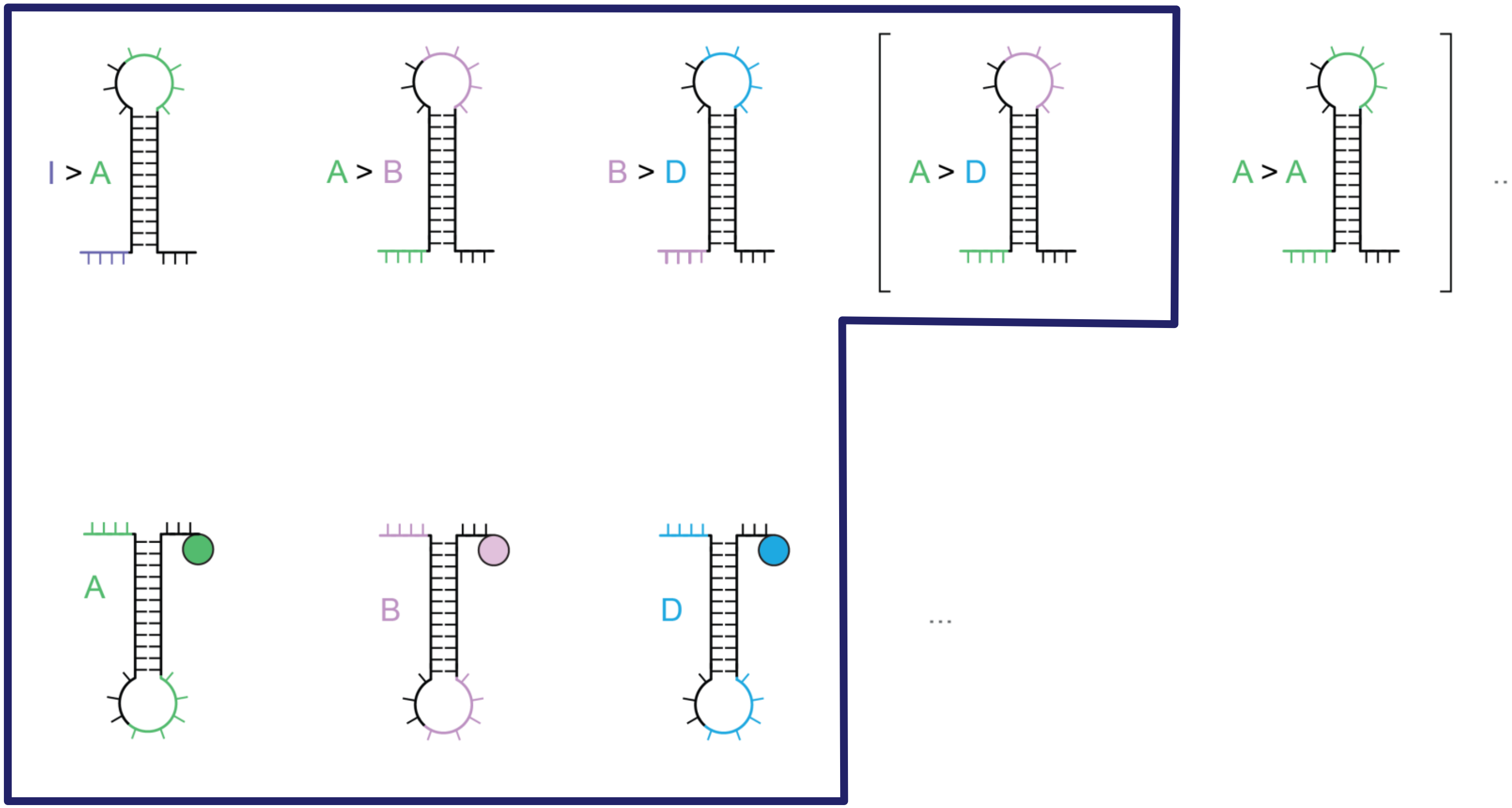
R.A. Muscat, M.L. McKee, P.J. Milnes, J. Bath, R.K. O'Reilly[3] A.J. Turberfield
Nature Chem. **8**, 542-548 (2016)

Non-deterministic program (branchpoint)



Make polymer A B D (● ● ●) or A D (● ●)

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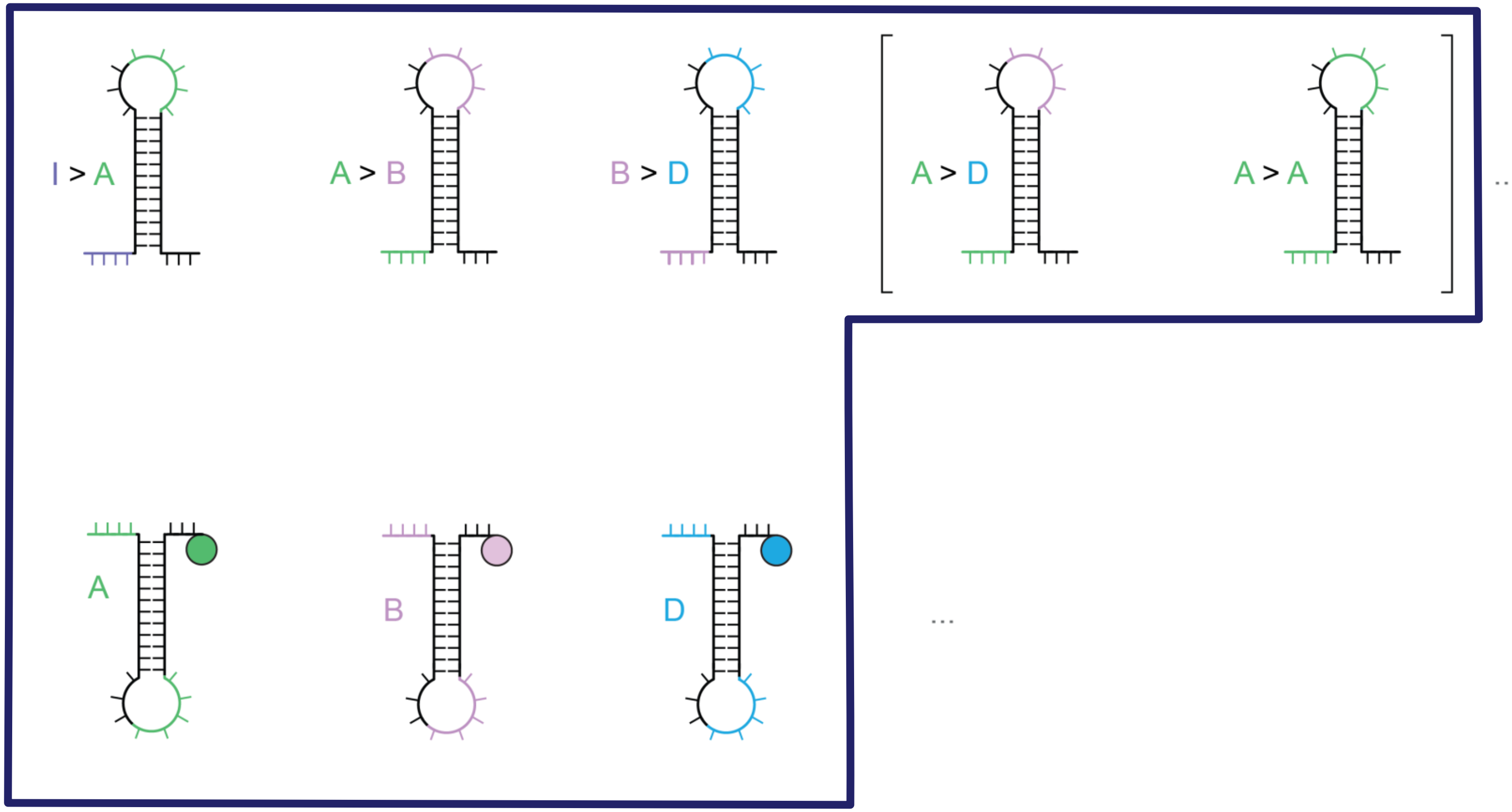
R.A. Muscat, M.L. McKee, P.J. Milnes, J. Bath, R.K. O'Reilly[3] A.J. Turberfield
Nature Chem. **8**, 542-548 (2016)

Non-deterministic program (branchpoint, indefinite loop)



Make polymer A_n B D or A_n D

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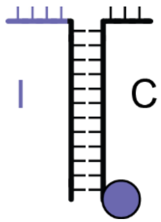
R.A. Muscat, M.L. McKee, P.J. Milnes, J. Bath, R.K. O'Reilly[3] A.J. Turberfield
Nature Chem. **8**, 542-548 (2016)

Reaction starts with an initiator **I** bound to the cargo **C**

The growing polymer chain is built on the cargo strand **C**



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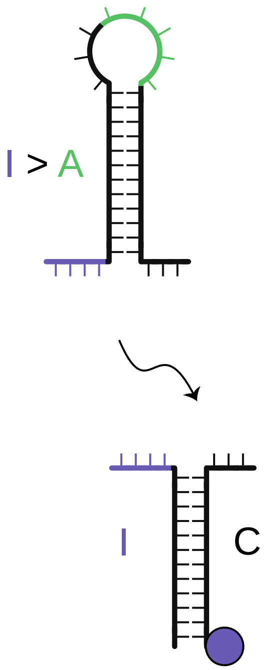
Nature Chem. **8**, 542-548 (2016)

The instruction **I > A** binds to **I•C**



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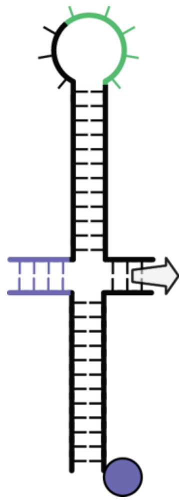
The hairpin **I** > **A** is opened by branch migration, **C** is transferred onto **I** > **A**



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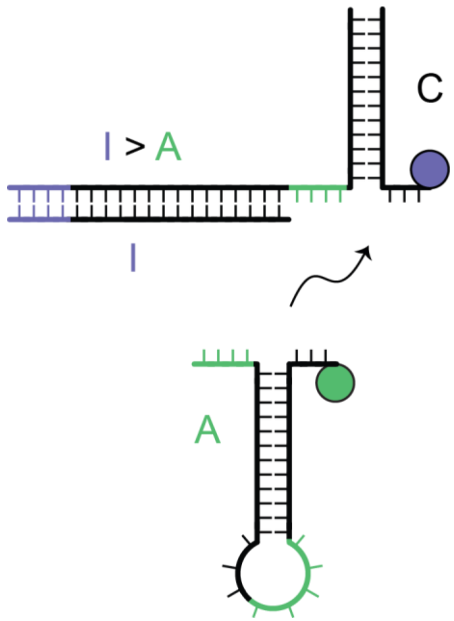
The hairpin **I** > **A** is opened by branch migration, **C** is transferred onto **I** > **A**

The chemistry hairpin **A** can bind to the open instruction hairpin



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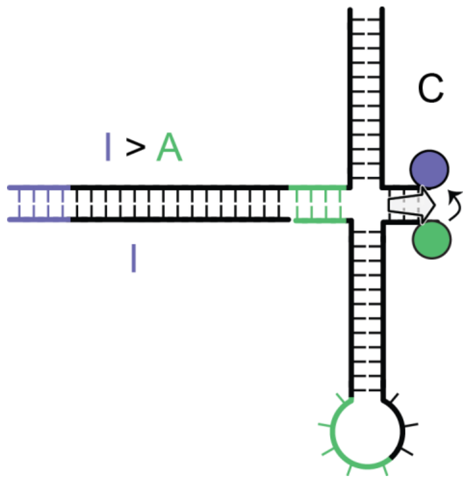
Once bound, the functional group **A** (●) is held close to C (●) and can react

At the same time, **A** is opened by branch migration and **C** is transferred onto **A**



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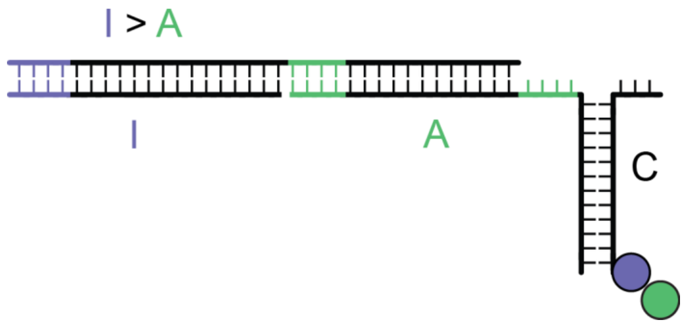
Building block **A** (●) is transferred to C (●)



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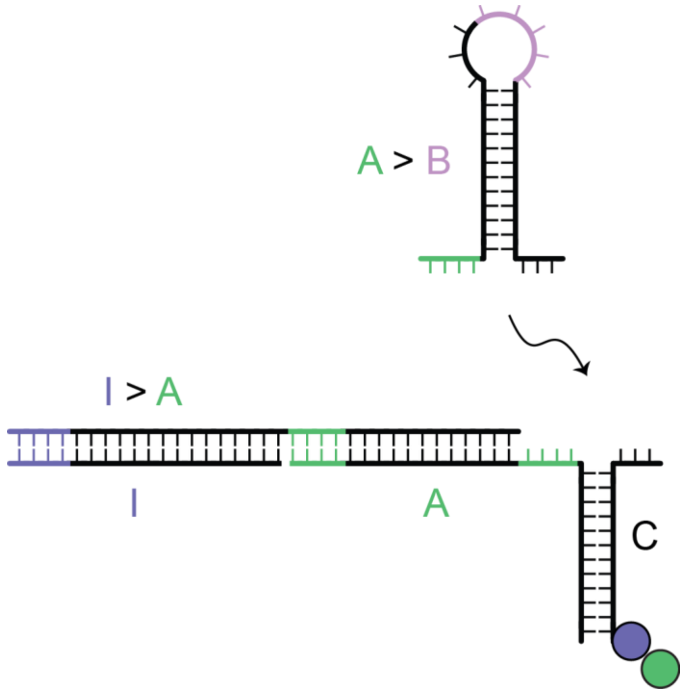
The next instruction, **A** > **B** binds



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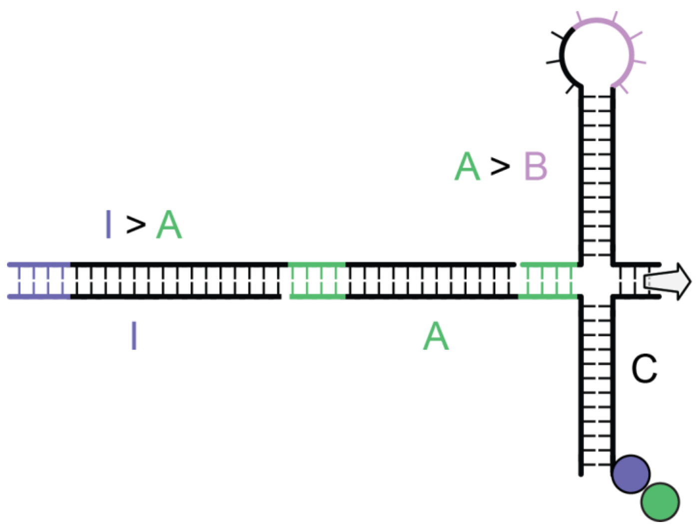

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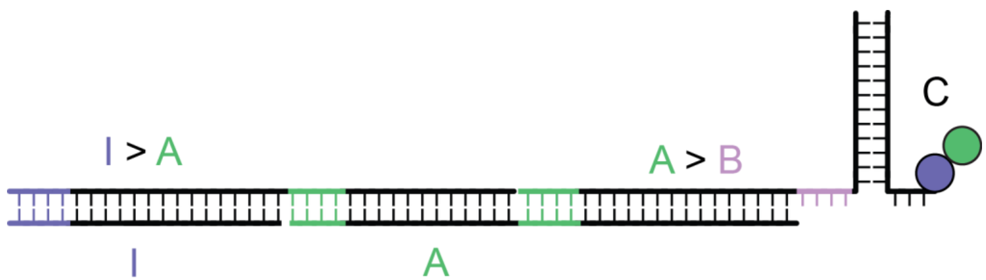
Nature Chem. **8**, 542-548 (2016)

A > B is opened by branch migration



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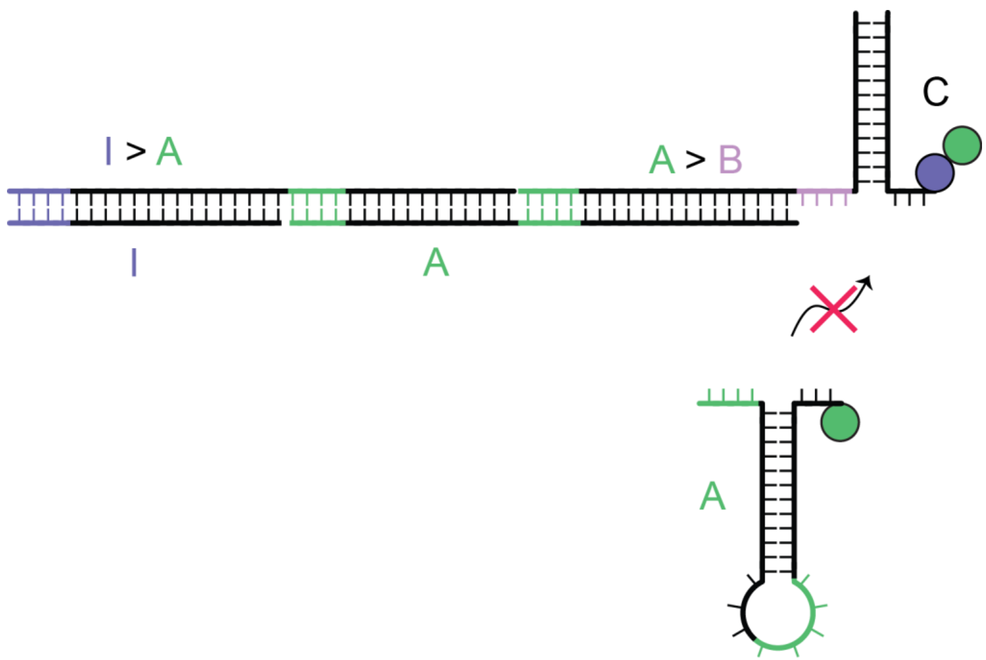
Nature Chem. **8**, 542-548 (2016)

The chemistry strand **A**, although present in solution, doesn't bind (toeholds don't match)



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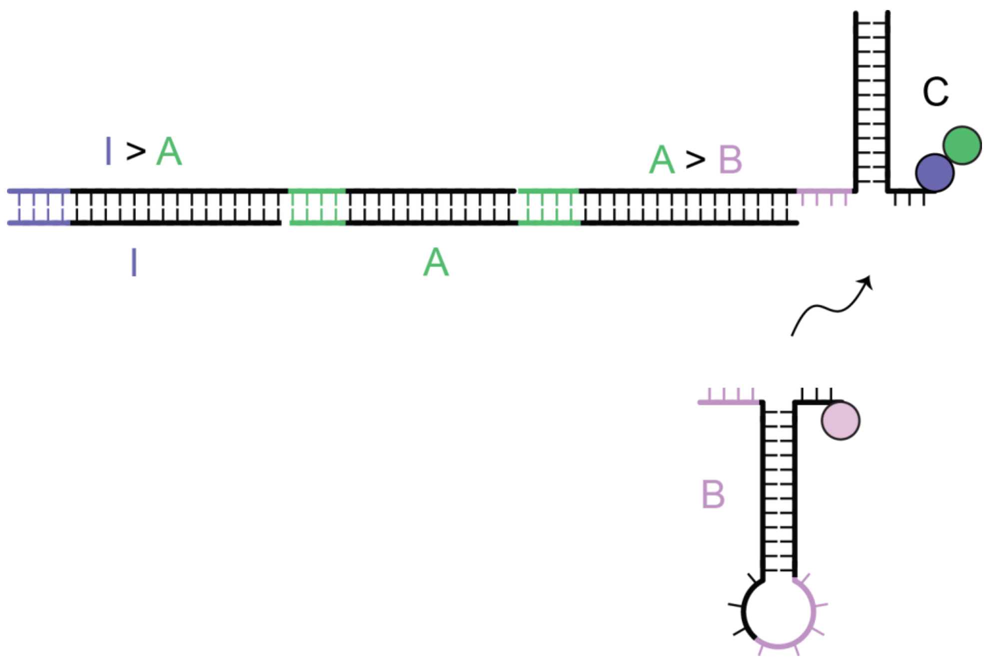
The chemistry strand B binds (toeholds match)



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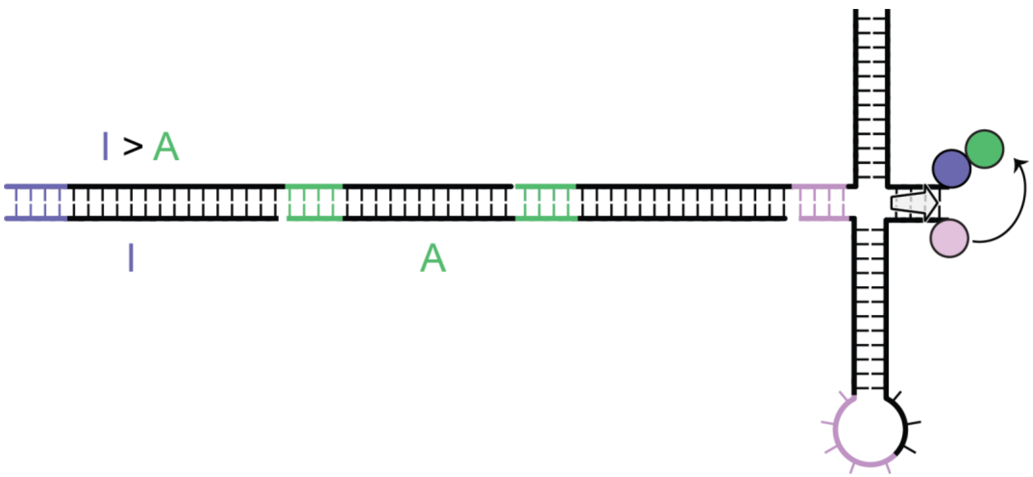
Nature Chem. **8**, 542-548 (2016)

Building block B (●) is transferred to A (●)



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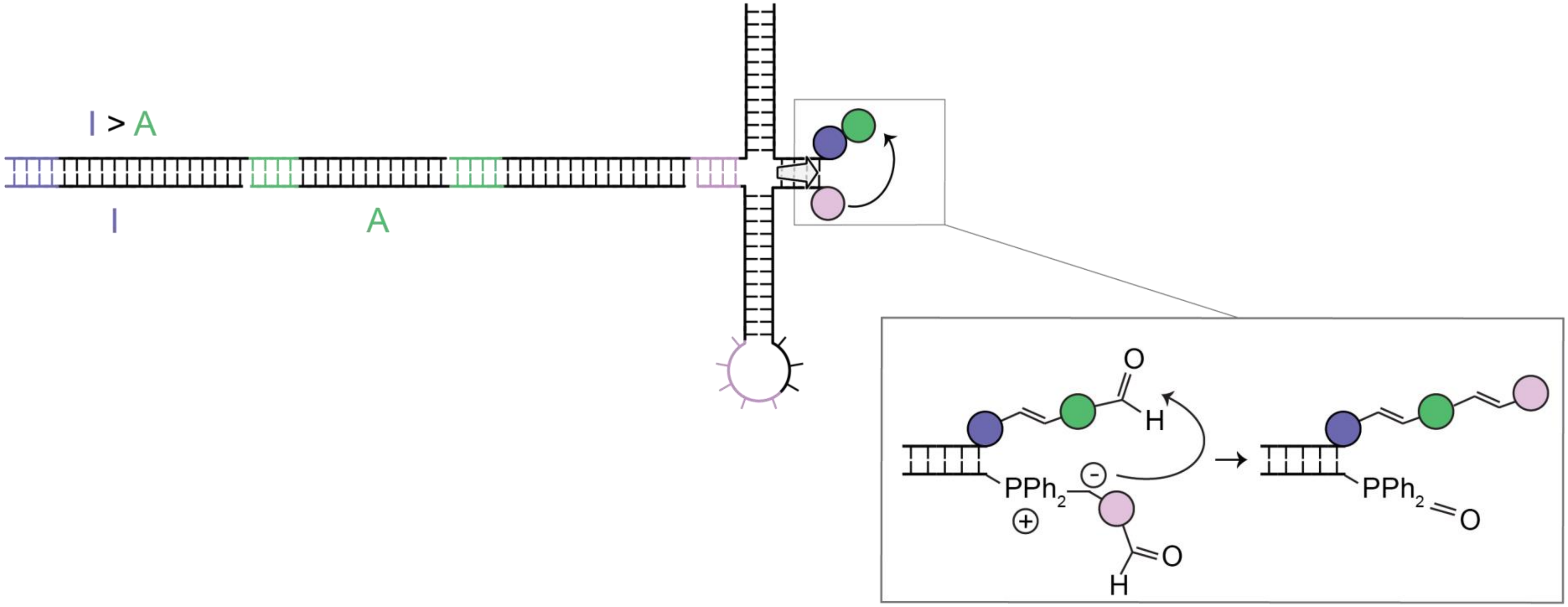
Building block B (●) is transferred to A (●)



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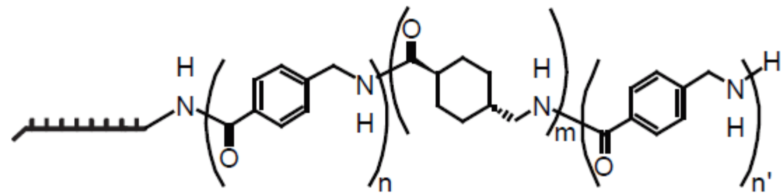
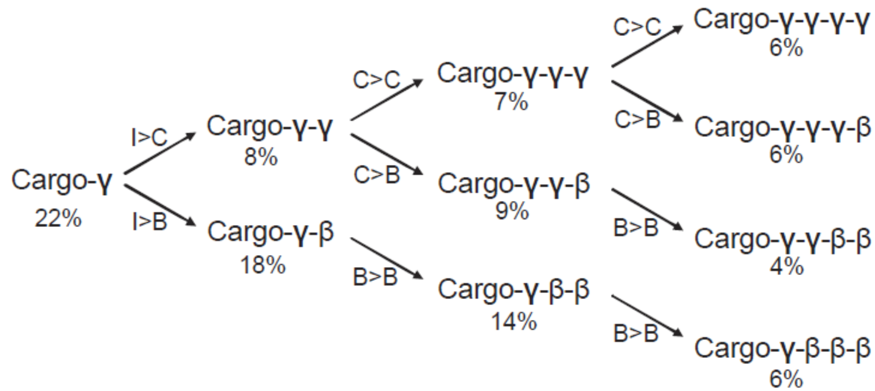
Autonomous combinatorial synthesis of a polypeptide

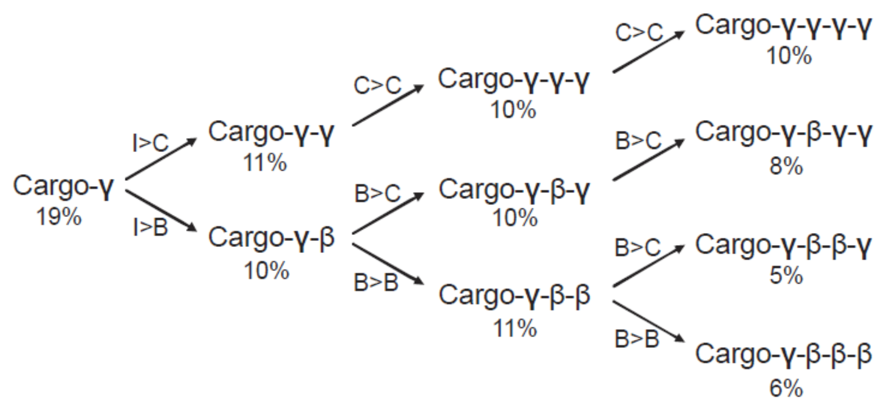


Cargo-$\gamma_n\beta_m\gamma_{n'}$ (n = 1-4, m = 0-3, n' = 0-2)

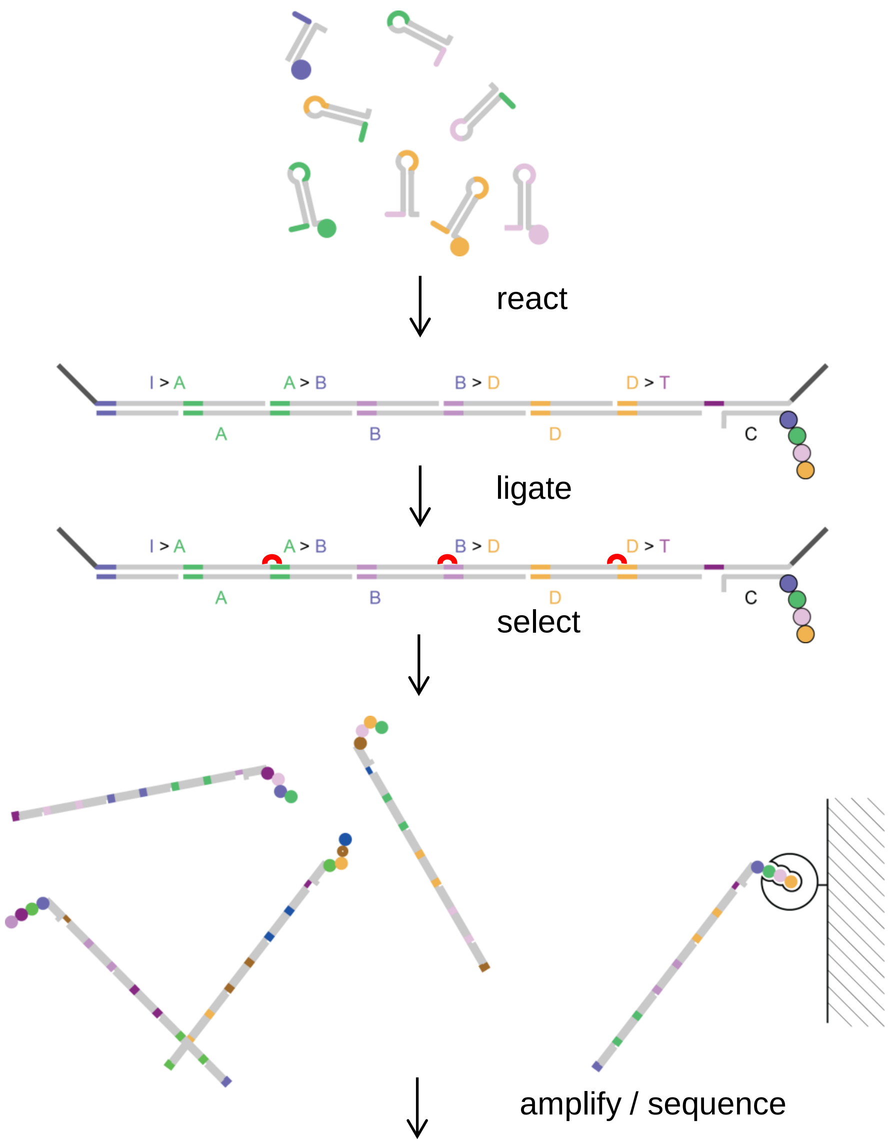
Reaction 1: I>B, B>B, C>C, I>C, C>B



Reaction 2: I>B, B>B, C>C, I>C, B>C



Ligation of one strand of the HCR duplex creates a permanent record



DNA-templated chemistry: selection and evolution

Use a DNA program to control oligomer synthesis; program determines reaction sequence (program is *gene*):

Generate library of programs	random insertions cut and shuffle
Synthesize product library	ensure product remains bound to program (*ribosome display)*
Select fittest products	usually by binding or cleaving
Amplify selected programs	polymerase chain reaction transform bacteria
Mutate	

So far –

- sequence-specific hybridization can be used to program the self-assembly of DNA and RNA nanostructures.

  nucleic acid nanostructures can ...

  - act as atomically precise scaffolds
  - exhibit programmed dynamic behaviour
  - control chemical reactions
  - compute
  - function *in vivo* (or fixed cells)

Given the ability to manufacture 3D structures with nm precision, and to create molecular systems that integrate sensing, computation and actuation –

what should we make?