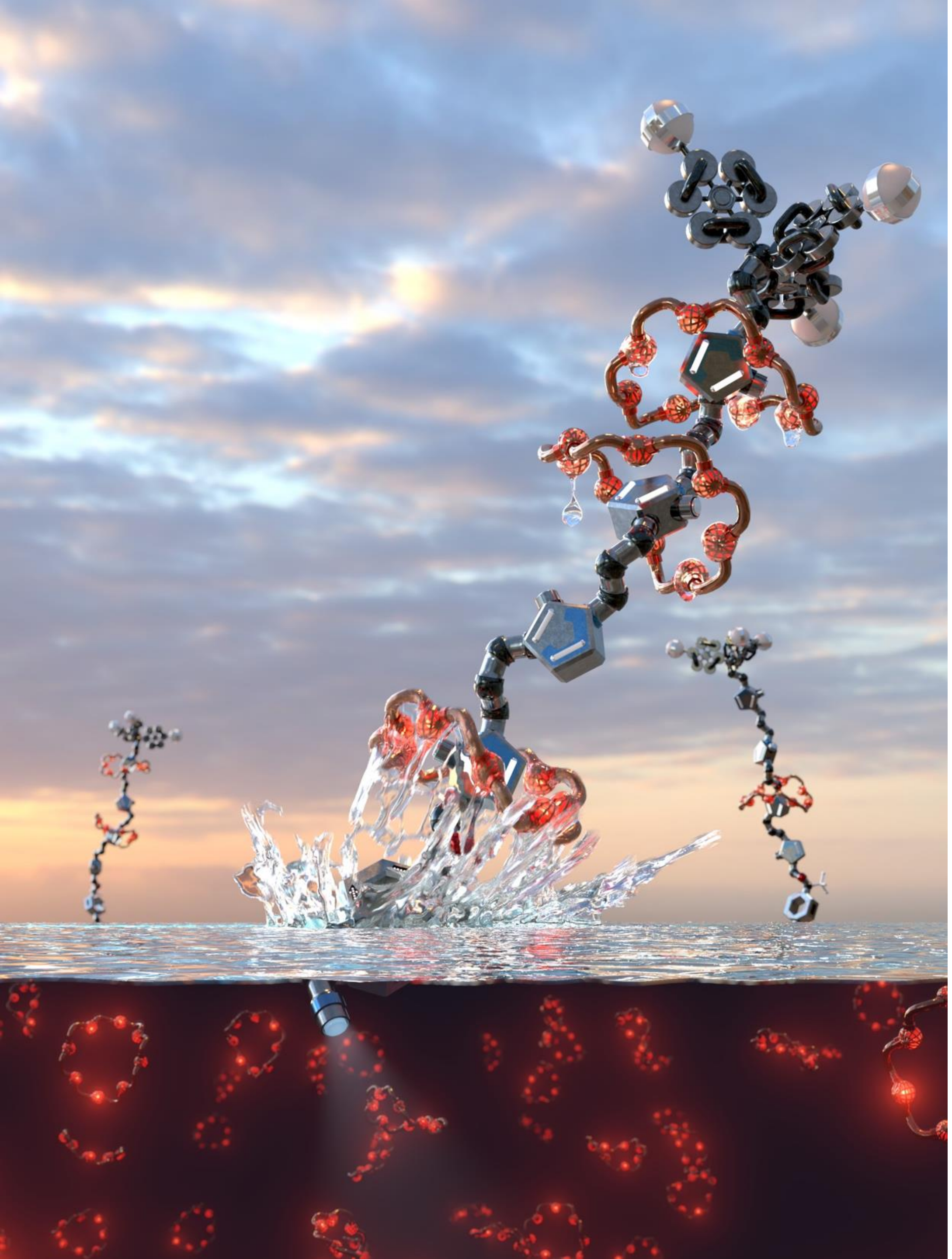


Synthesis and Analysis of Life-like Systems out of Equilibrium

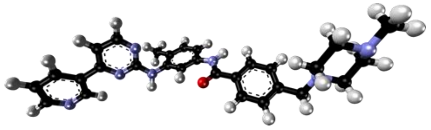
25th April 2025

Shuntaro Amano

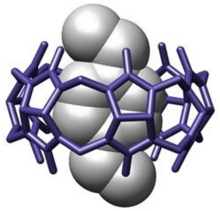
@Non-equilibrium thermodynamics workshop, Edinburgh

Why Out-of-Equilibrium Systems?

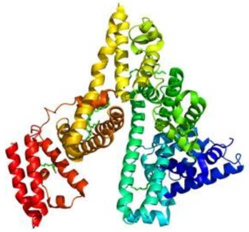
Equilibrium systems



Small molecule

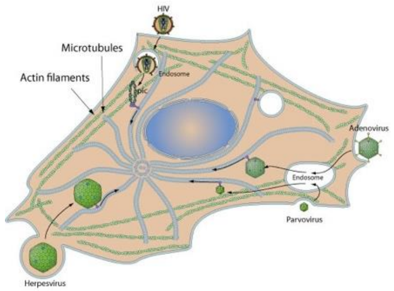


Supramolecular structure

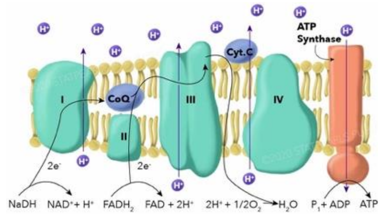


Polymer

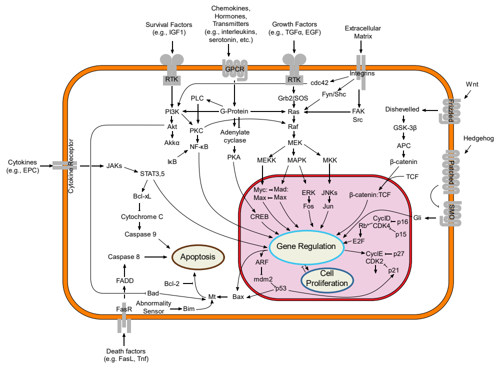
Out-of-equilibrium systems



Transportation



Energy conversion

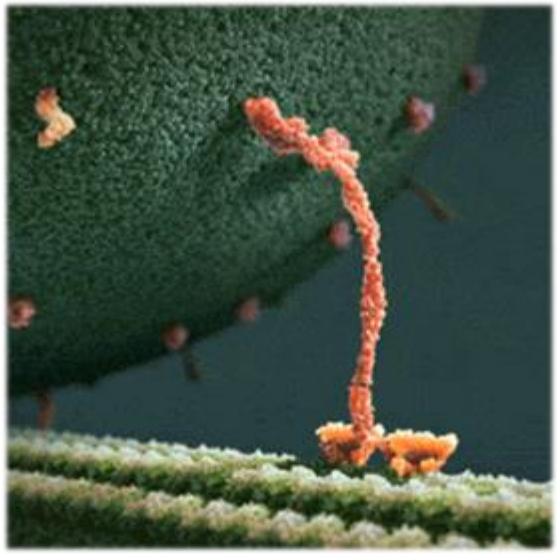


Response to signals

Functional systems inspired by out-of-equilibrium processes in biology

Biology Uses Molecular Machines

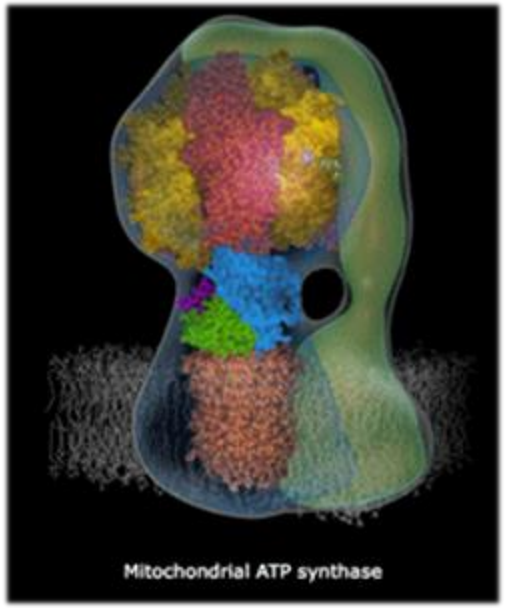
- Kinesin



Transport cargos inside cells

Responsible for mitosis, axonal transport etc.

- ATP synthase

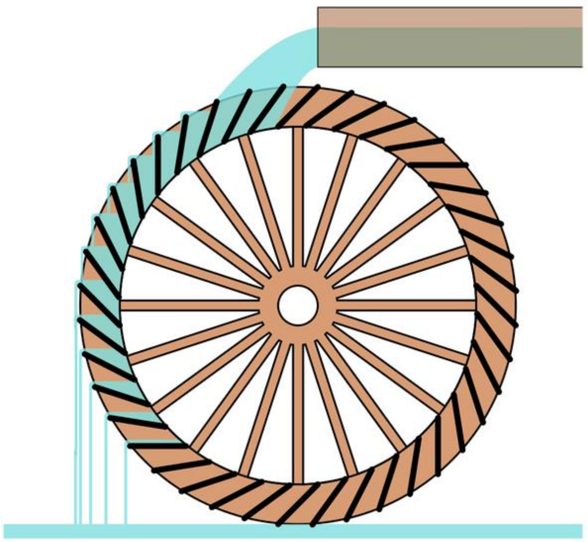


Synthesize ATP from ADP and phosphate

High efficiency (nearly 100%)

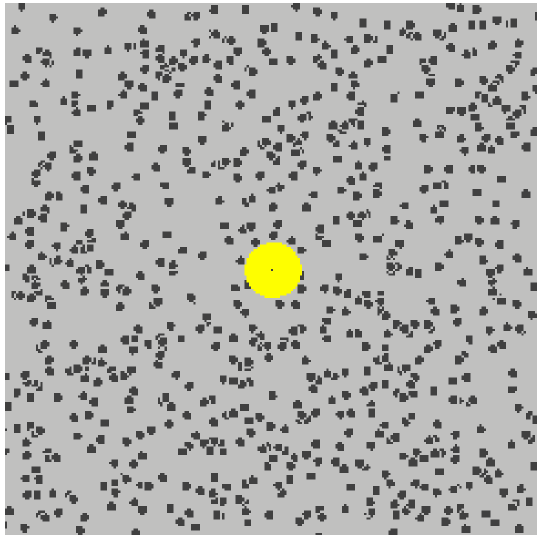
Macroscopic and Microscopic Worlds are Different

Macroscopic world



- Dominant factors: gravity, inertia
- Water wheel, electric motor

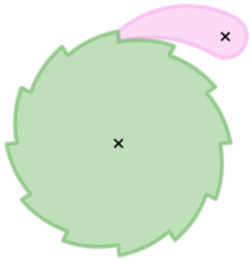
Microscopic world

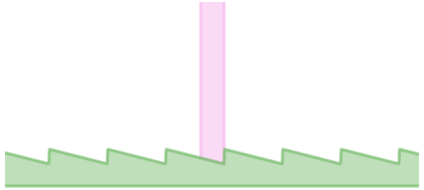


- Dominant factors: viscosity, Brownian motion
- Brownian ratchet mechanisms

Astumian, *Phys. Chem. Chem. Phys.* **9,** 5067-5083 (2007).

Brownian Ratchet Mechanisms





Animations by Arglin Kamplin

Brownian ratchets rectify Brownian motion to achieve directional motion

Key questions

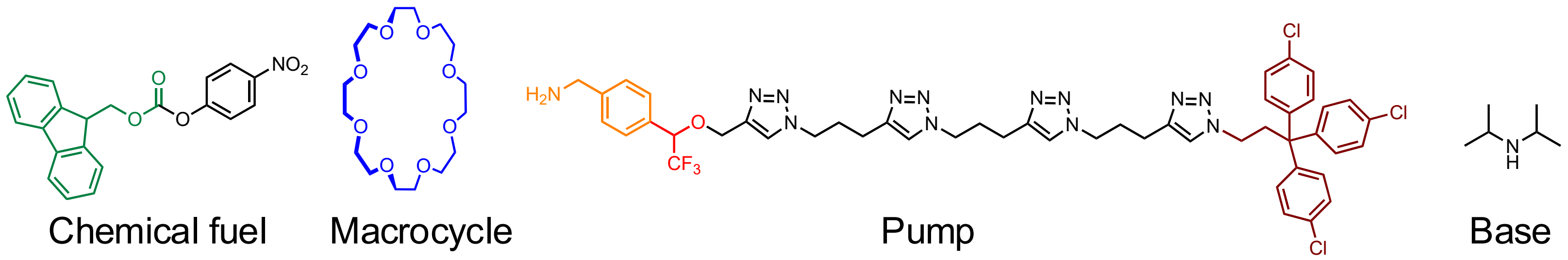
- New synthetic Brownian ratchets?
- General design strategy?
- Application to systems other than molecular machines?

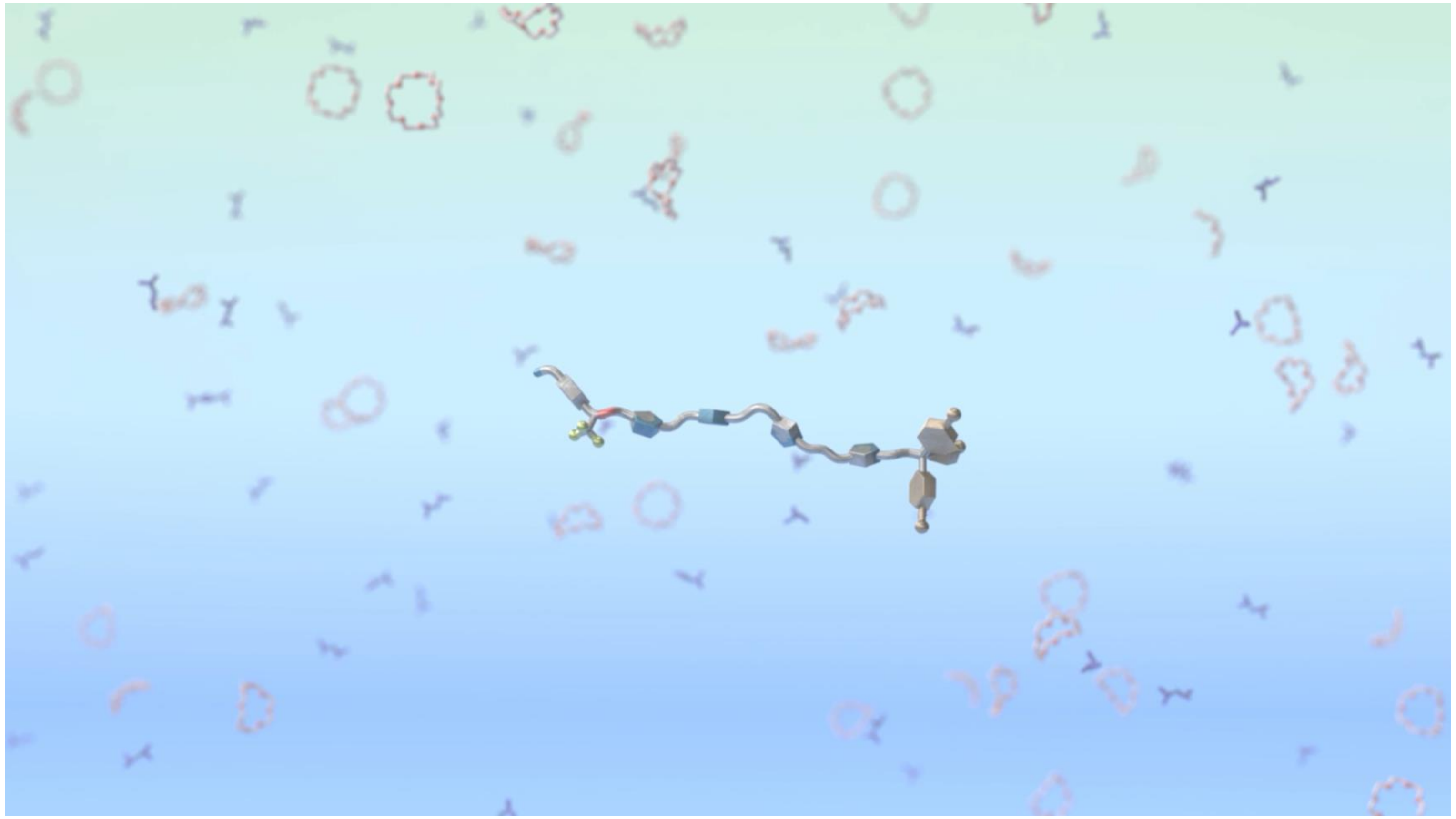
Theory **Experiment**

Sangchai *et al.*, *Angew. Chem. Int. Ed.* **62**, e202309501 (2023)

Borsley *et al.*, *Angew. Chem. Int. Ed.* **63**, e202400495 (2024)

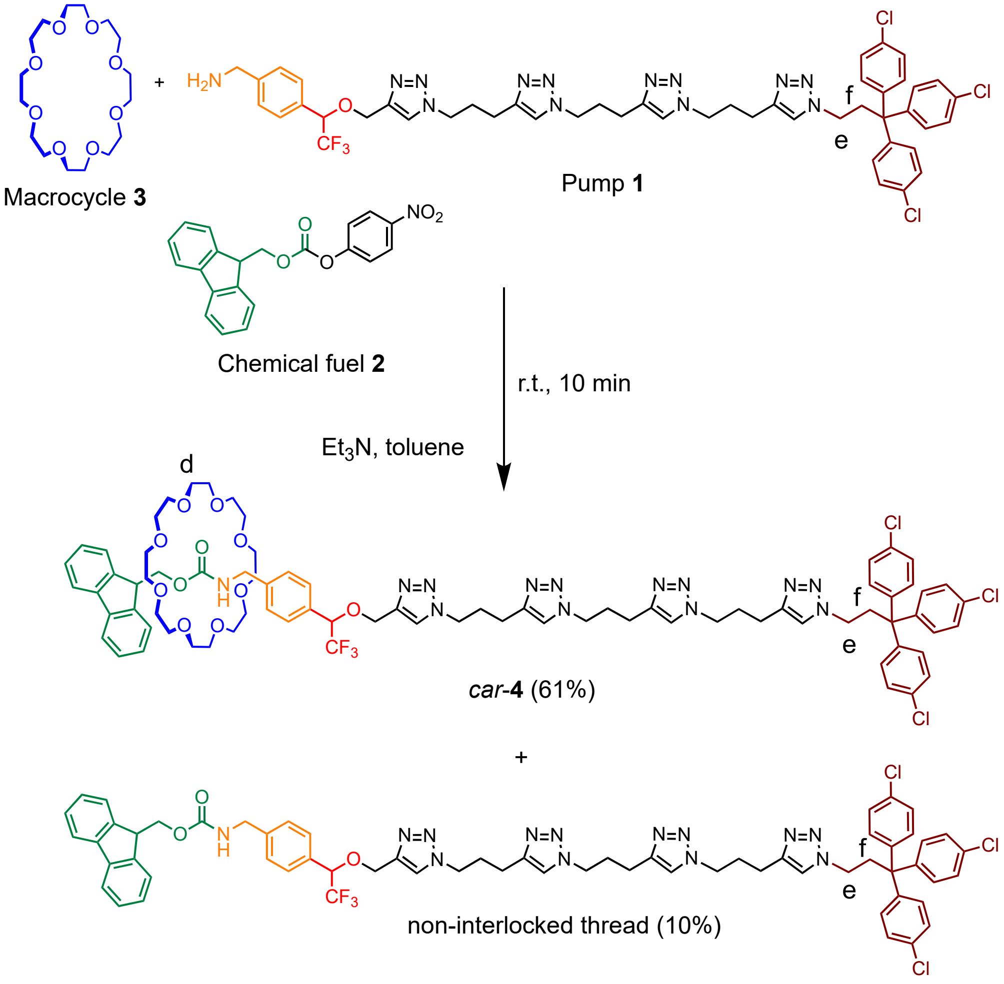
New Brownian Ratchet: Autonomous Molecular Pump

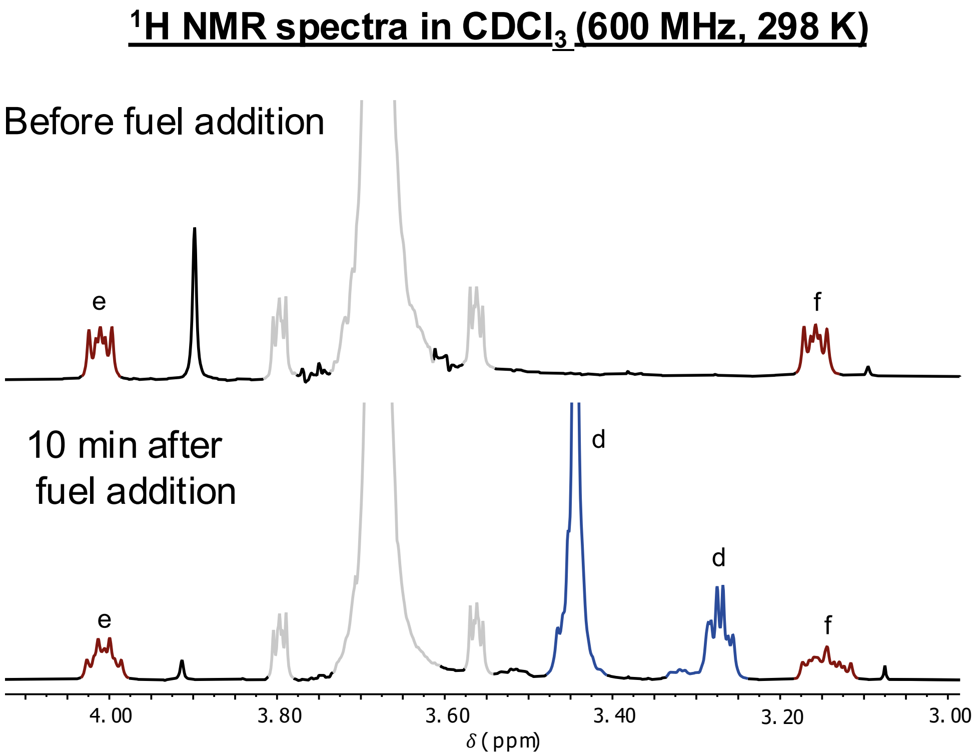




Amano *et al.*, *Nature* **594,** 529–534 (2021)

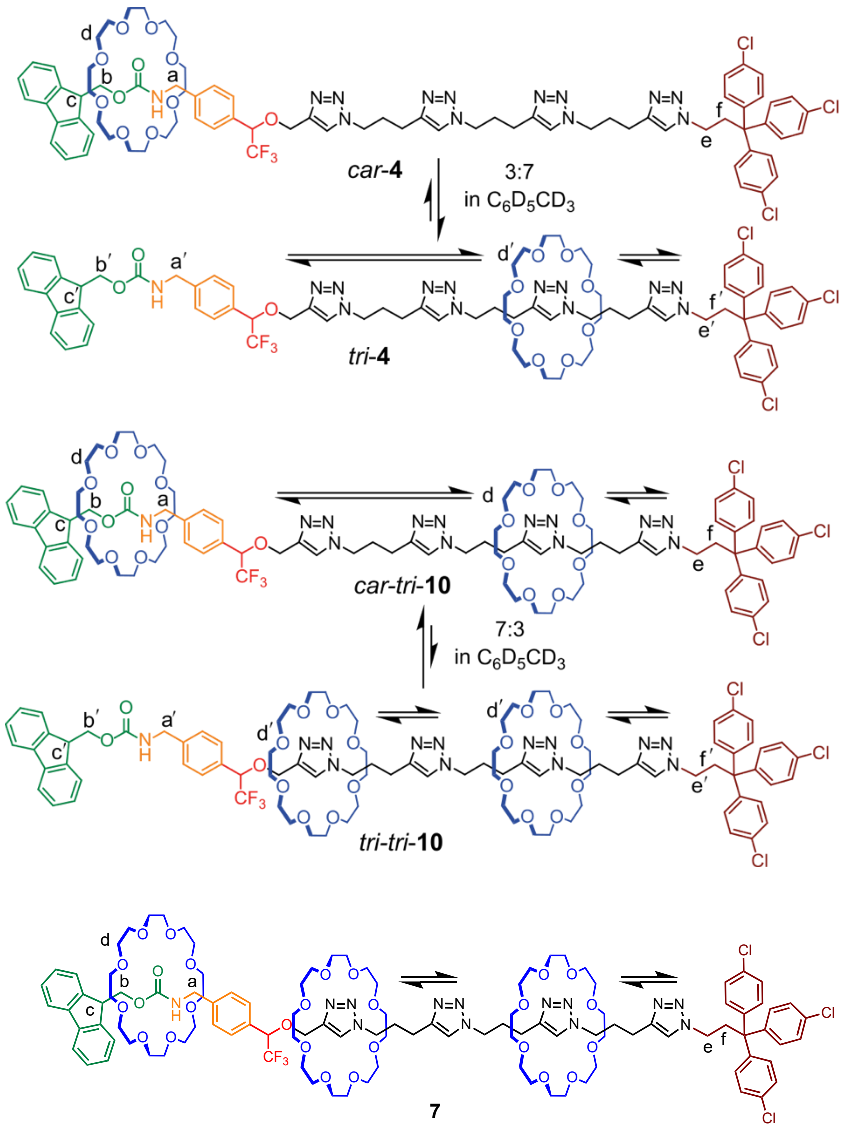
Macrocycle Take-up Study

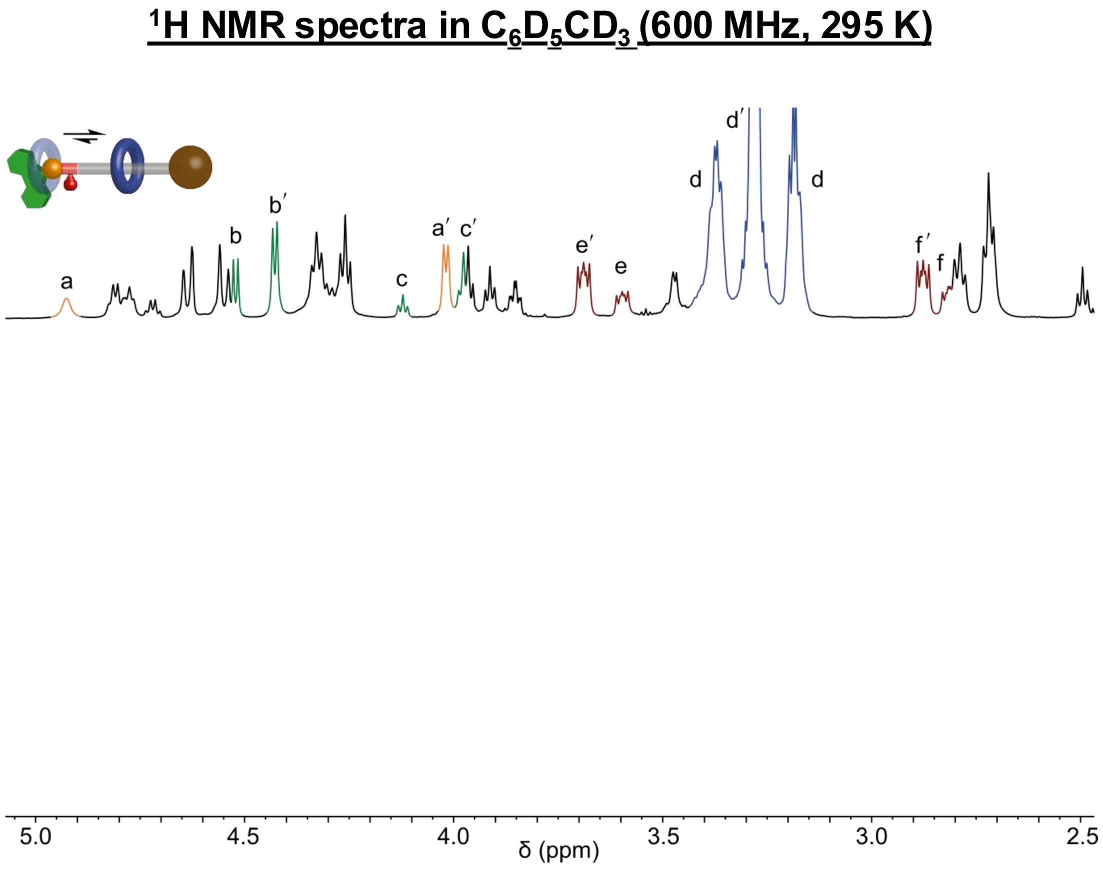




Amano *et al.*, *Nature* **594,** 529–534 (2021)

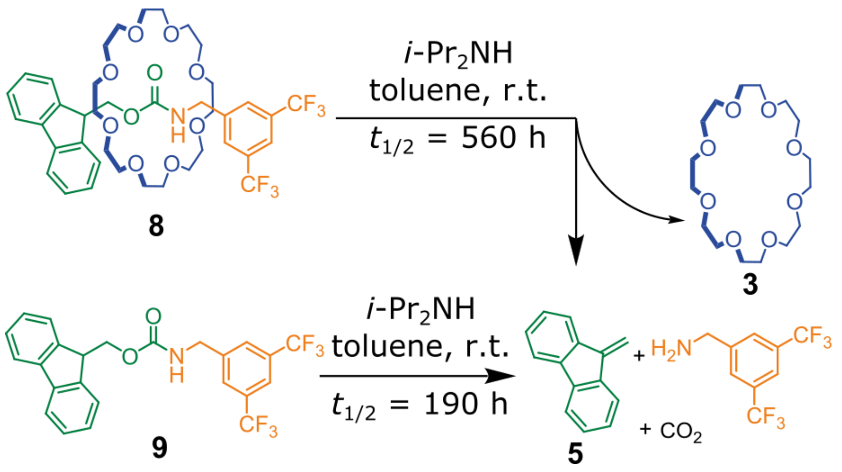
Macrocycle Displacement Study

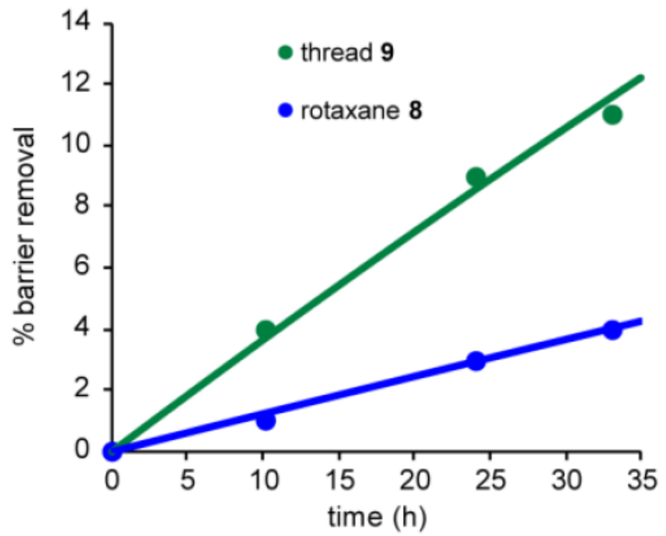


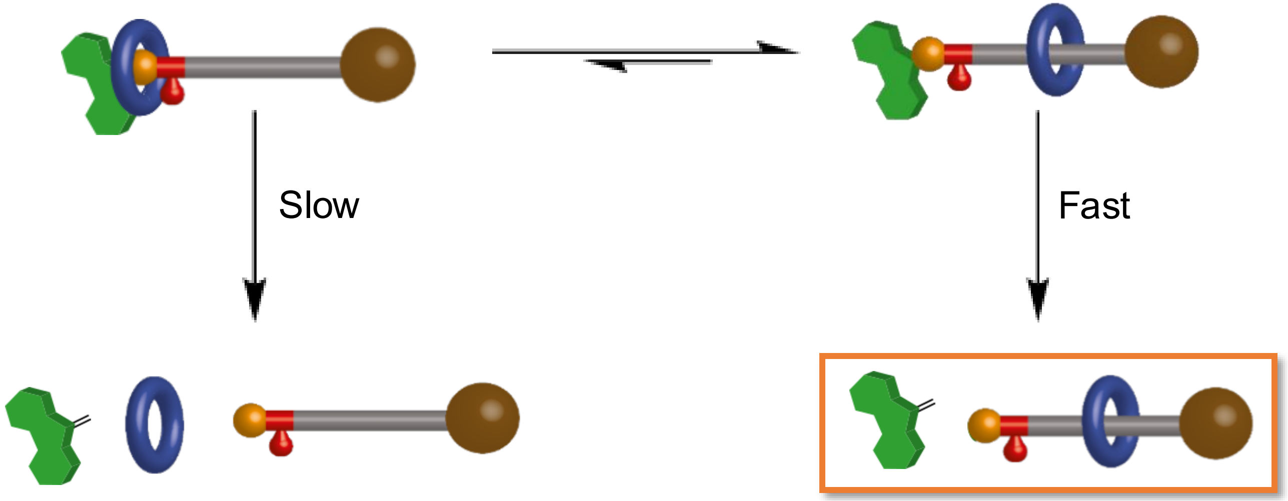


Amano *et al.*, *Nature* **594,** 529–534 (2021)

Stropper Removal Study

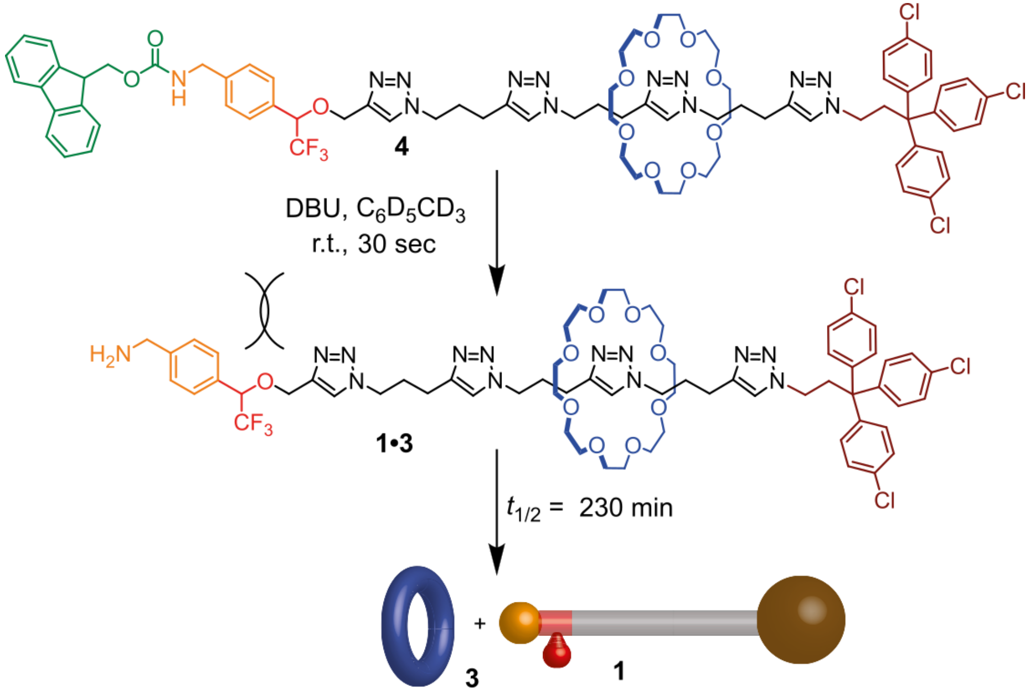


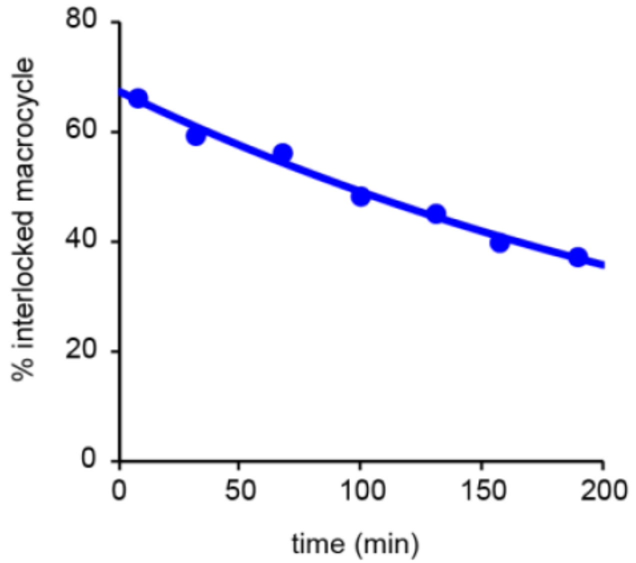


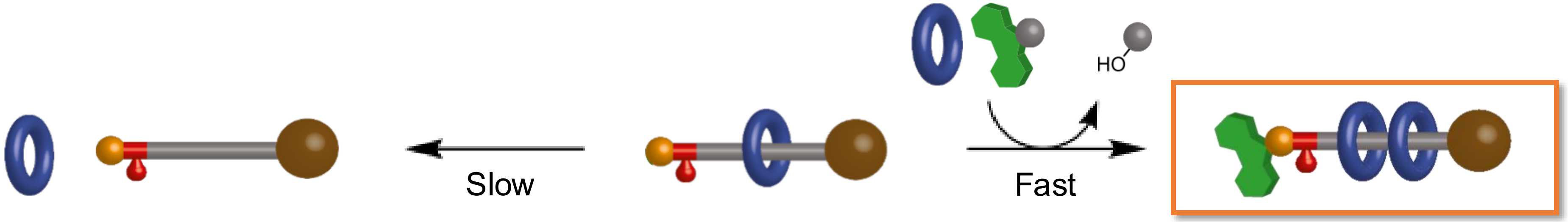


Amano *et al.*, *Nature* **594,** 529–534 (2021)

Dethreading Study

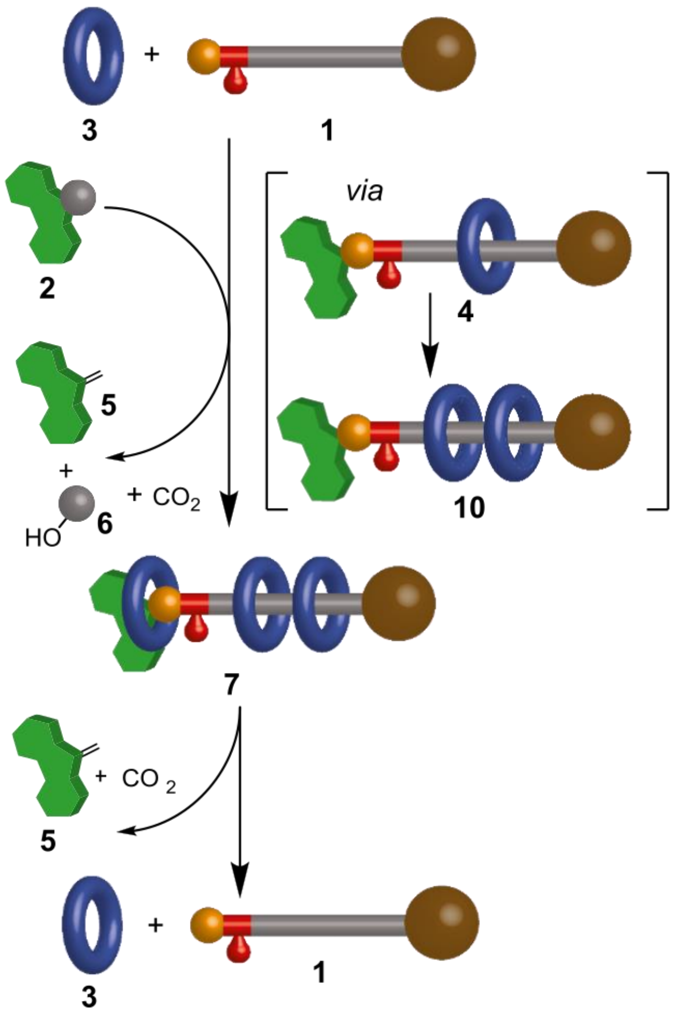


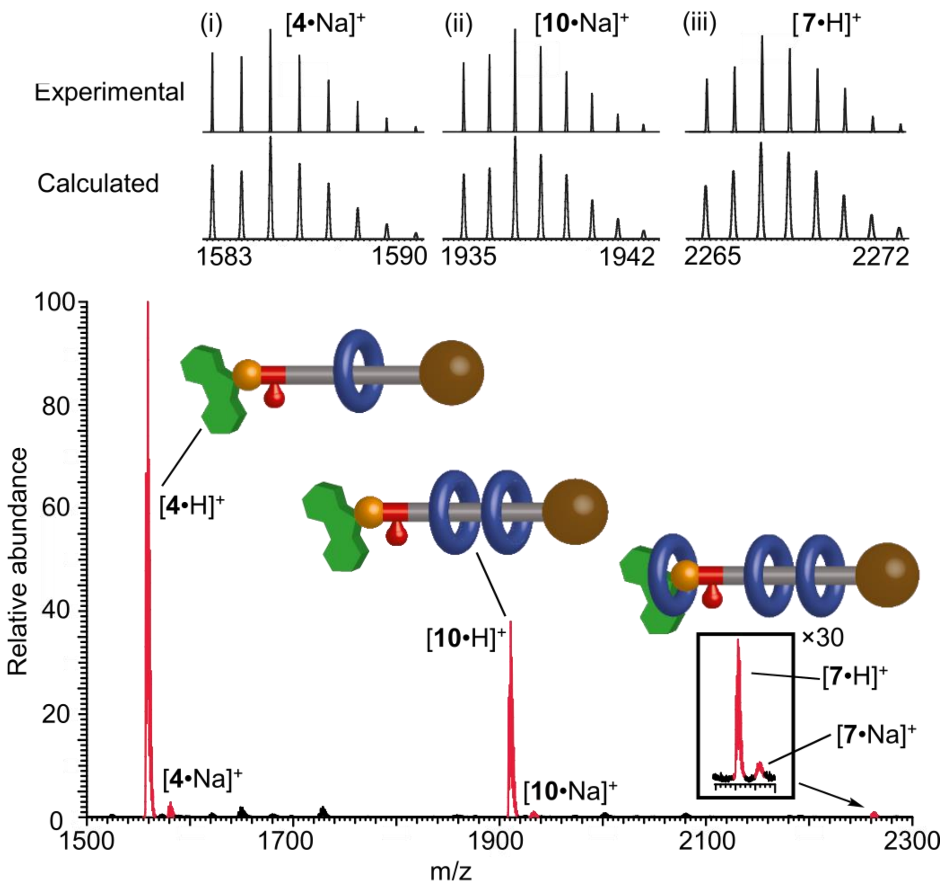




Amano *et al.*, *Nature* **594,** 529–534 (2021)

Confirmation of Multi-cycle Pumping



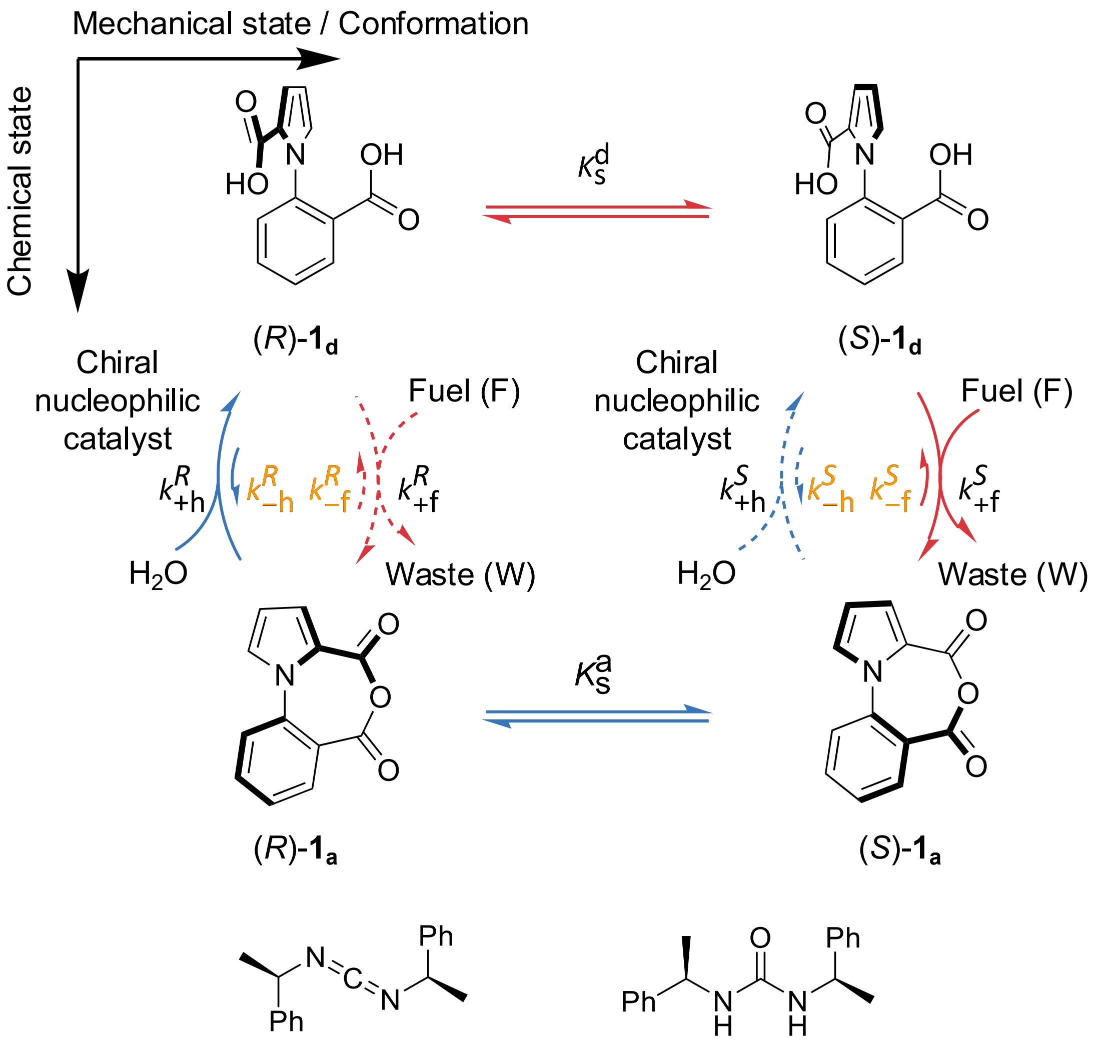


Simple Design Principle?

Operation Conditions: *i*-Pr_2NH, toluene, r.t., 16 h

Amano *et al.*, *Nature* **594,** 529–534 (2021)

Kinetic Asymmetry Shows Directionality, but Complicated

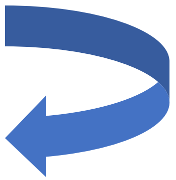


Carbodiimide fuel (F)

Urea waste (W)

$$K_r = \frac{([F]k^S_{+f} + k^S_{-h})}{([F]k^R_{+f} + k^R_{-h})} \times \frac{([W]k^R_{-f} + [H_2O]k^R_{+h})}{([W]k^S_{-f} + [H_2O]k^S_{+h})} \times K_s^d \times K_s^a$$

$K_r > 1$



$K_r < 1$

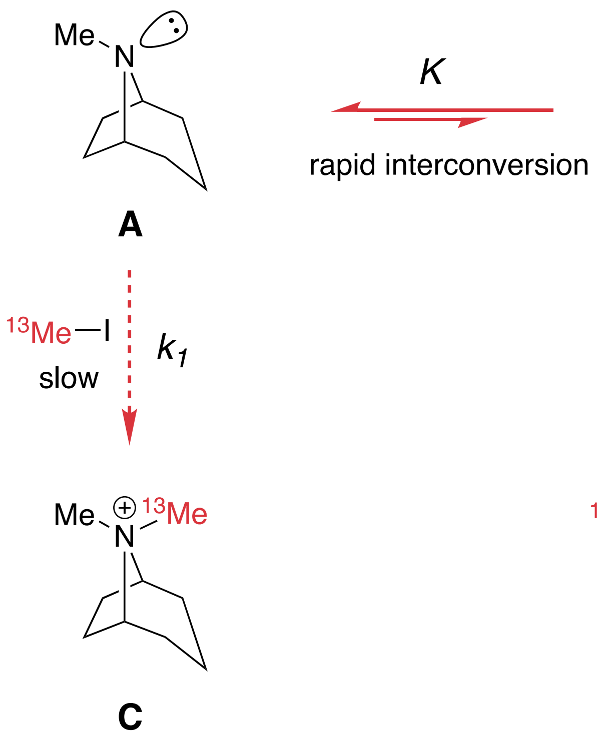
$K_r = 1$ ❌ No directional motion

Astumian, *Nat. Commun.*, 2019, **10**, 3837.

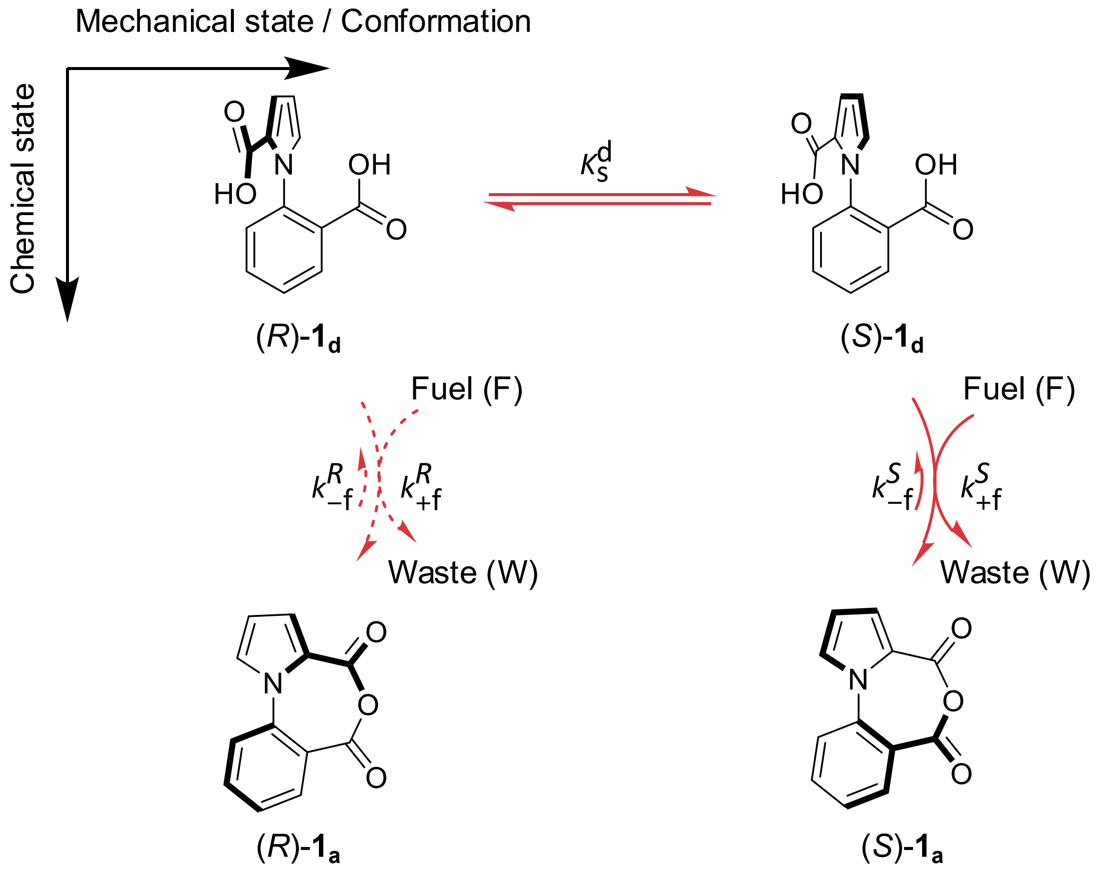
Amano *et al.*, *J. Am. Chem. Soc.* **144**, 20153–20164 (2022)

Simpler Principle: Curtin–Hammett Asymmetry Factor (F_{C-H})

- Curtin–Hammett Principle



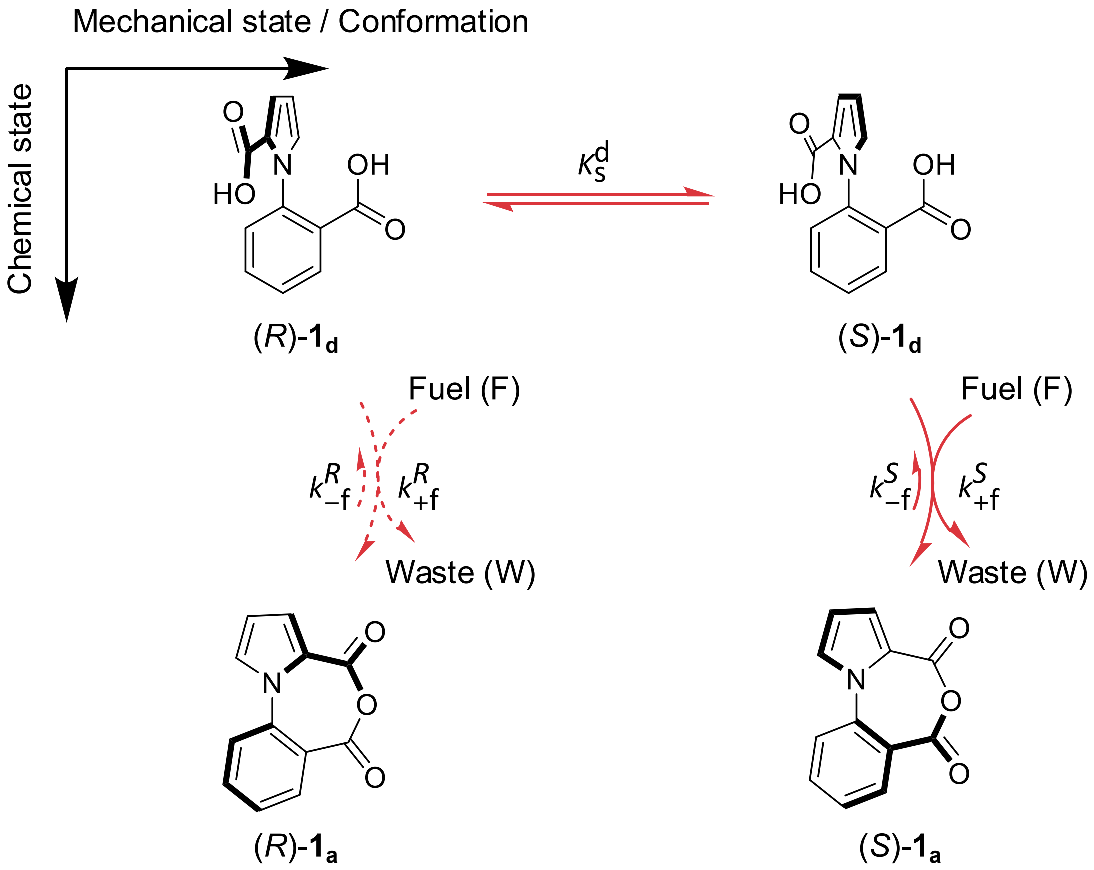
$$\frac{[\boldsymbol{D}]}{[\boldsymbol{C}]} \approx \frac{k_2}{k_1} K$$

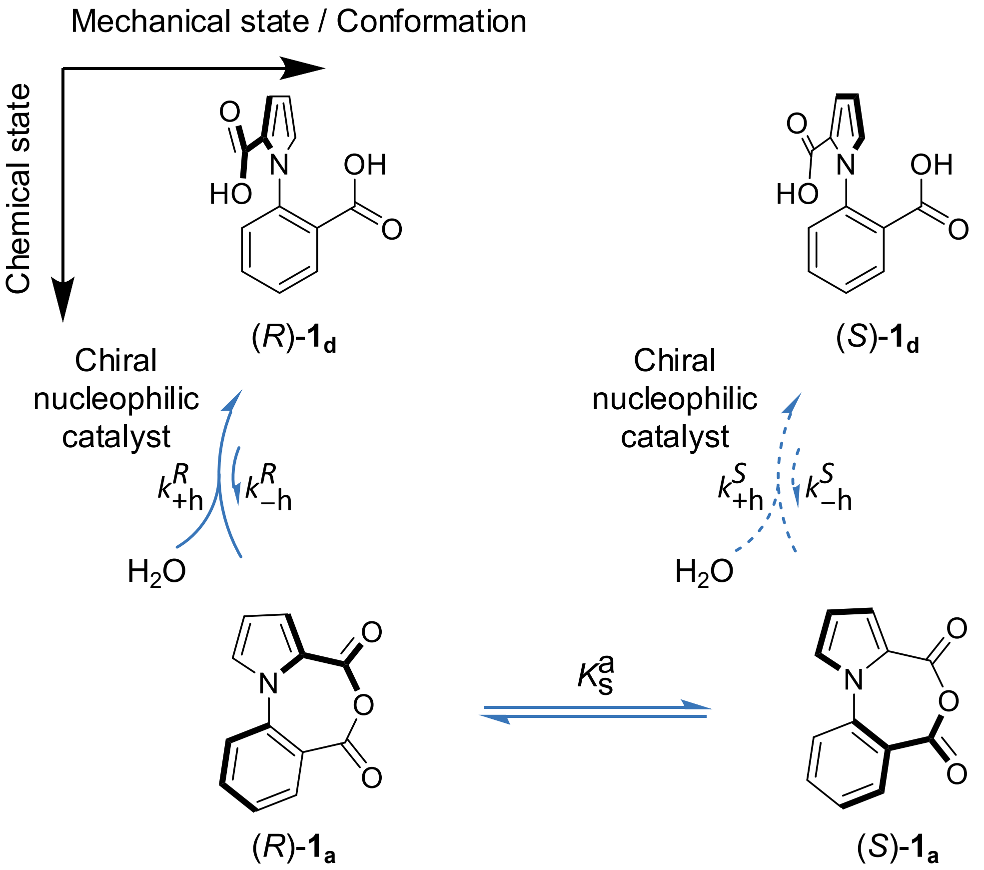


$$\frac{[(S)-\mathbf{1}_a]}{[(R)-\mathbf{1}_a]} \approx \frac{k_{+f}^S}{k_{+f}^R} K_s^d$$

Seeman, *Chem. Rev.*, 1983, **83**, 83–134. Otzenberger *et al.*, *J. Org. Chem.*, 1974, **39**, 319–321.

Simpler Principle: Curtin–Hammett Asymmetry Factor ($F_{C\text{–}H}$)

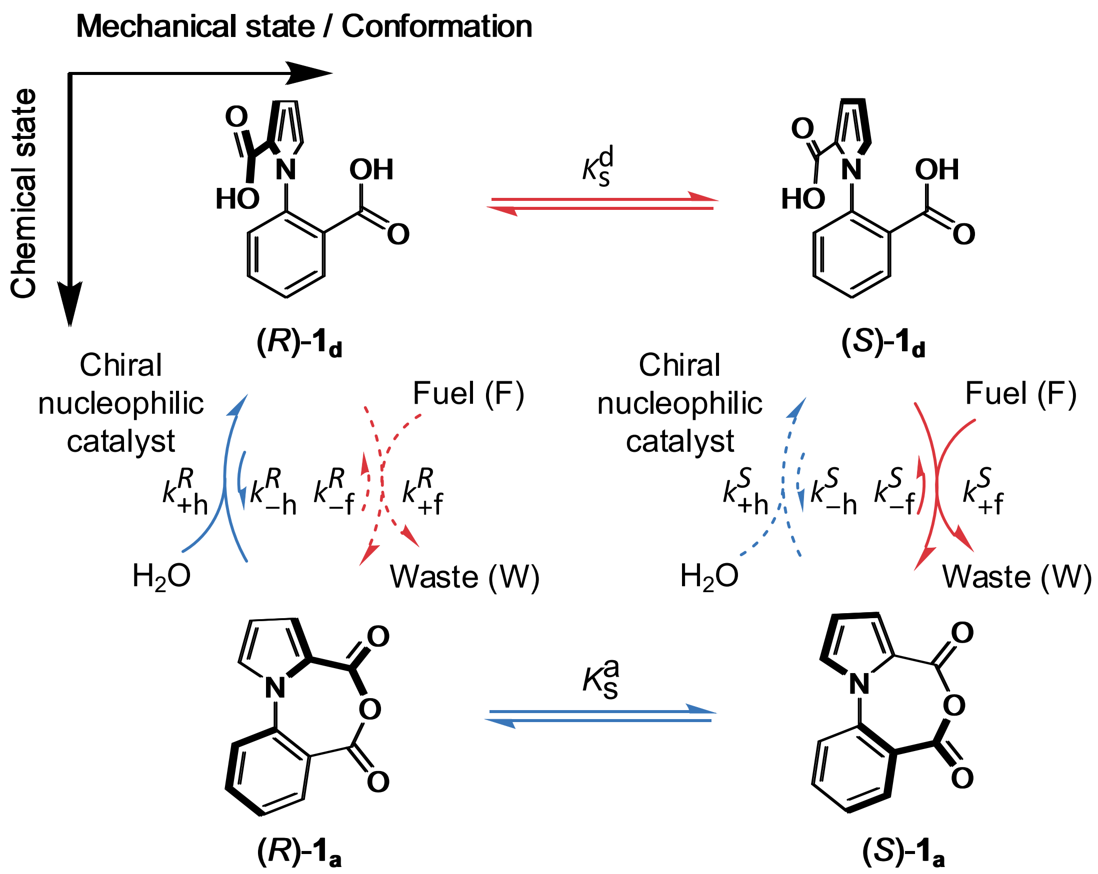




$$\frac{[(S)-\mathbf{1}_a]}{[(R)-\mathbf{1}_a]} \approx \frac{k^S_{+f}}{k^R_{+f}} K_s^d \qquad\qquad \frac{[(R)-\mathbf{1}_d]}{[(S)-\mathbf{1}_d]} \approx \frac{k^R_{+h}}{k^S_{+h}} K_s^a$$

Amano *et al.*, *J. Am. Chem. Soc.* **144**, 20153–20164 (2022)

Simpler Principle: Curtin–Hammett Asymmetry Factor ($F_{C–H}$)

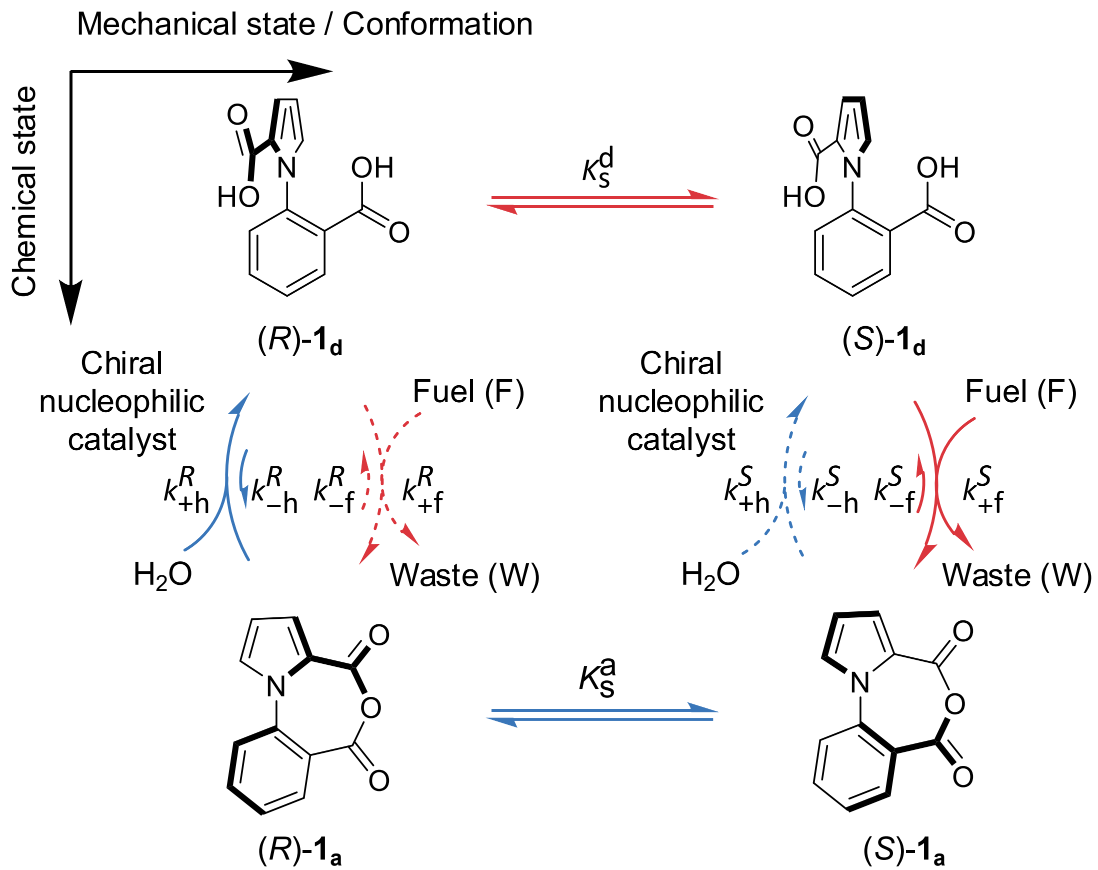


$$\frac{[(S)-\mathbf{1}_a]}{[(R)-\mathbf{1}_a]} \approx \frac{k^S_{+f}}{k^R_{+f}} K^d_s$$

$$\frac{[(R)-\mathbf{1}_d]}{[(S)-\mathbf{1}_d]} \approx \frac{k^R_{+h}}{k^S_{+h}} K^a_s$$

Amano *et al.*, *J. Am. Chem. Soc.* **144**, 20153–20164 (2022)

Simpler Principle: Curtin–Hammett Asymmetry Factor ($F_{C–H}$)

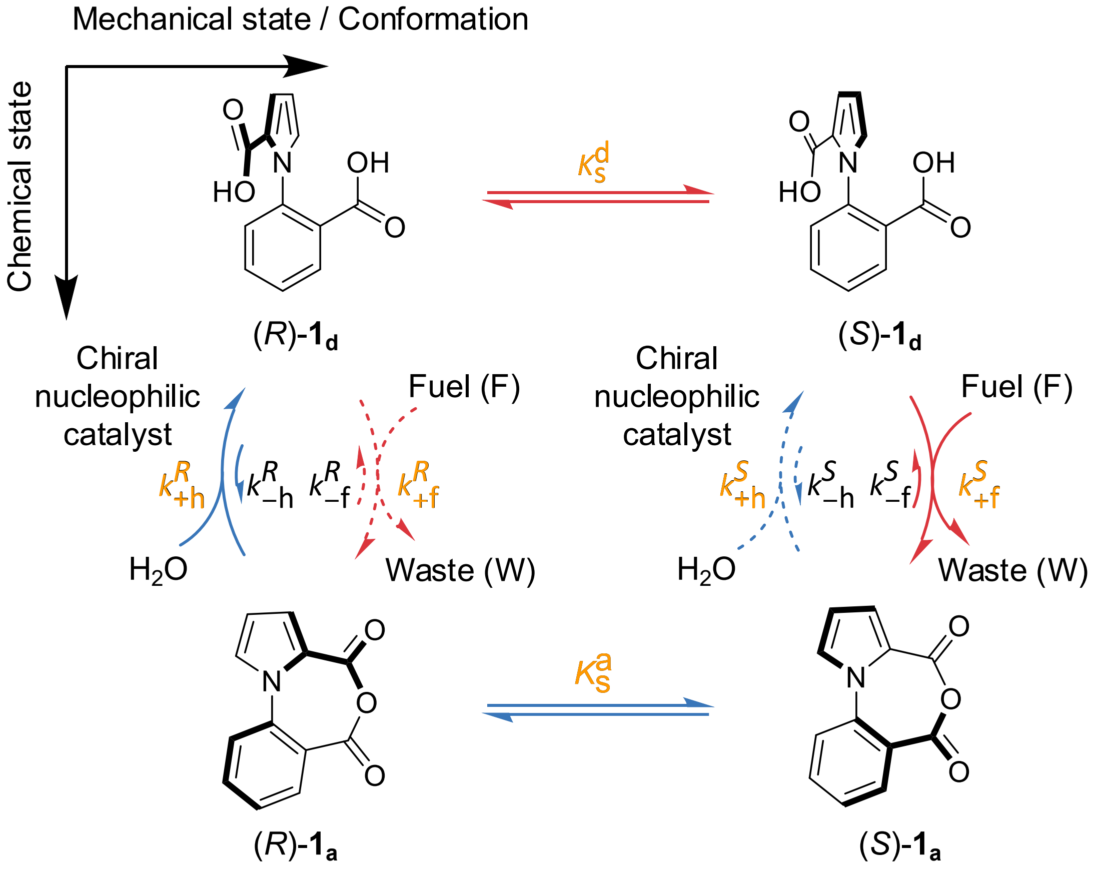


$$\frac{[(S)-\mathbf{1}_a]}{[(R)-\mathbf{1}_a]} \approx \frac{k_{+f}^{S}}{k_{+f}^{R}} K_s^d$$

$$\frac{[(R)-\mathbf{1}_d]}{[(S)-\mathbf{1}_d]} \approx \frac{k_{+h}^{R}}{k_{+h}^{S}} K_s^a$$

Amano *et al.*, *J. Am. Chem. Soc.* **144**, 20153–20164 (2022)

Simpler Principle: Curtin–Hammett Asymmetry Factor (F_{C-H})

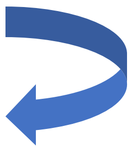


$$\frac{k_{+f}^S}{k_{+f}^R} K_S^d$$

$$\times\ \frac{k_{+h}^R}{k_{+h}^S} K_S^a$$

$$\frac{k_{+f}^S k_{+h}^R}{k_{+f}^R k_{+h}^S} K_S^d K_S^a \equiv F_{C-H}$$

$F_{C-H} > 1$



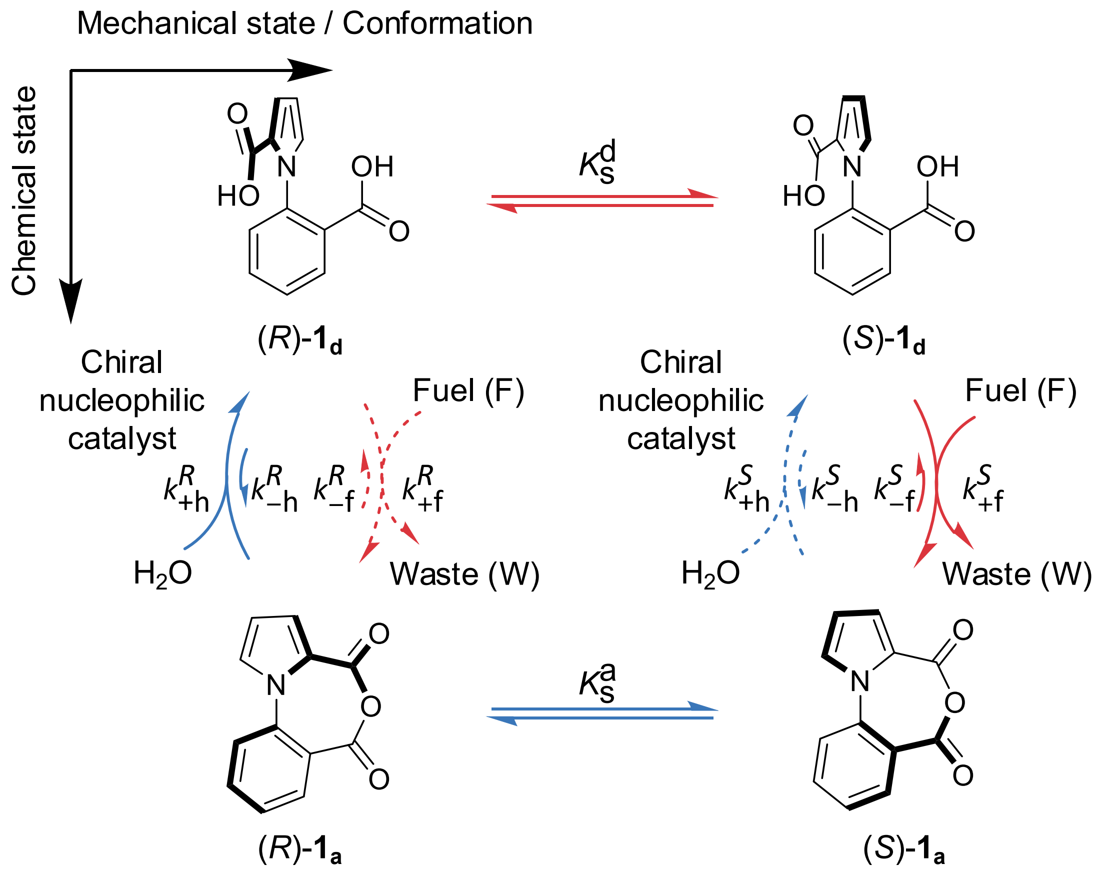
$F_{C-H} < 1$

$F_{C-H} = 1$ ❌ No directional motion

F_{C-H} shows directionality, and simpler

Amano *et al.*, *J. Am. Chem. Soc.* **144**, 20153–20164 (2022)

Two Design Elements for Directionality

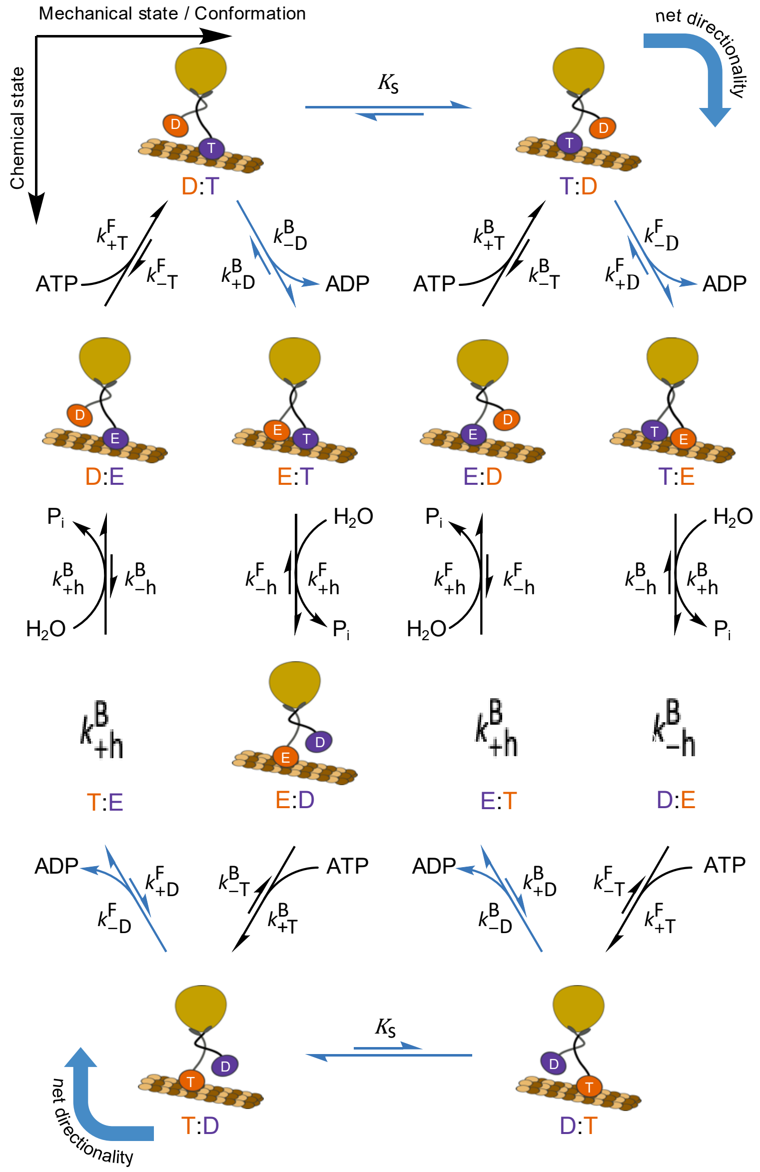


Chemical gating *Power stroke*

$$\frac{k_{+f}^{S} k_{+h}^{R}}{k_{+f}^{R} k_{+h}^{S}} K_s^d K_s^a \equiv F_{C-H}$$

Power strokes contribute to directionality
BUT
do not determine it

Power Strokes in a Biological Molecular Machine



Chemical gating *Power stroke*

$$F_{C-H}^{(kin)} = \frac{k_{+T}^{F} k_{+h}^{B} k_{-D}^{F}}{k_{+T}^{B} k_{+h}^{F} k_{-D}^{B}} K_s$$

$$k_{+T}^{F} \approx k_{+T}^{B}, k_{+h}^{B} \approx k_{+h}^{F}, k_{-D}^{F} \approx k_{-D}^{B}$$

$$F_{C-H}^{(kin)} \approx K_s \approx 1.25 \times 10^{6}$$

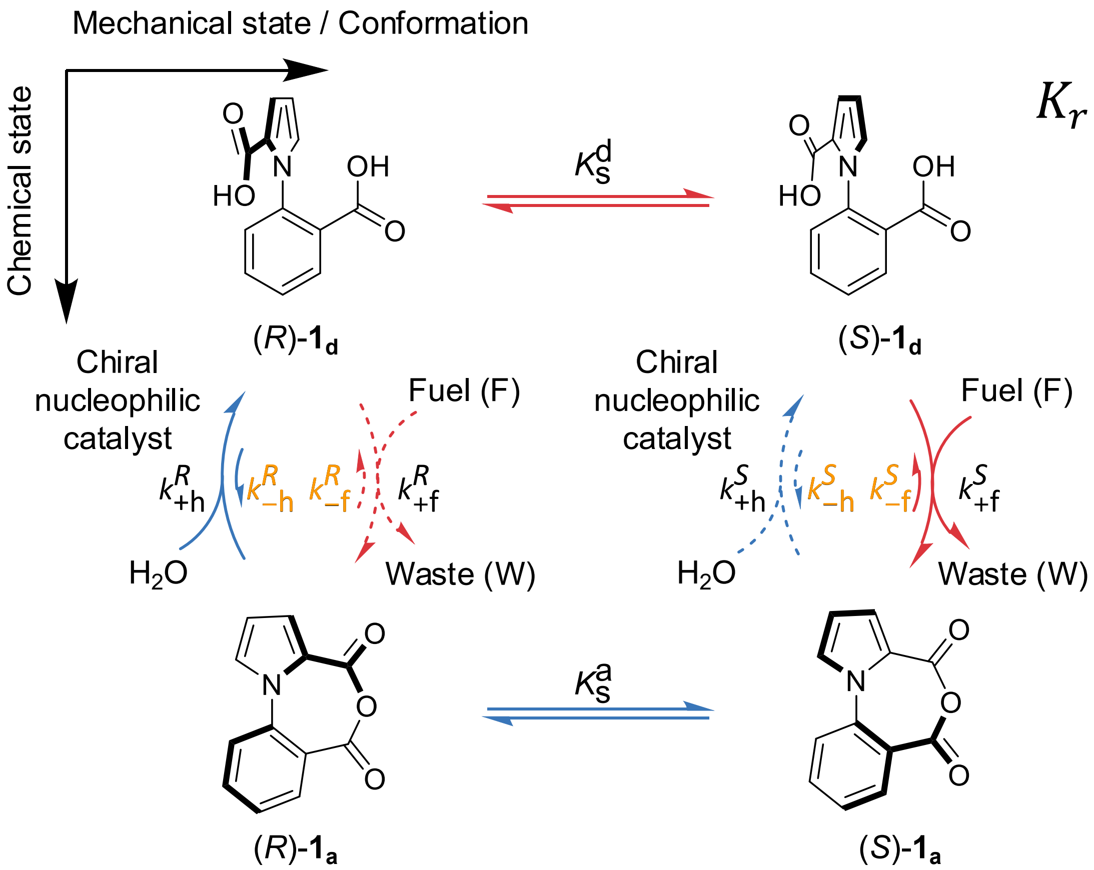
Power strokes are a dominant factor

Liepelt and Lipowsky, *Phys. Rev. Lett.* **98**, 258102 (2007).

Schief *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 1183–1188 (2004).

Carter and Cross, *Nature* **435**, 308–312 (2005).

Connection between K_r and F_{C-H}



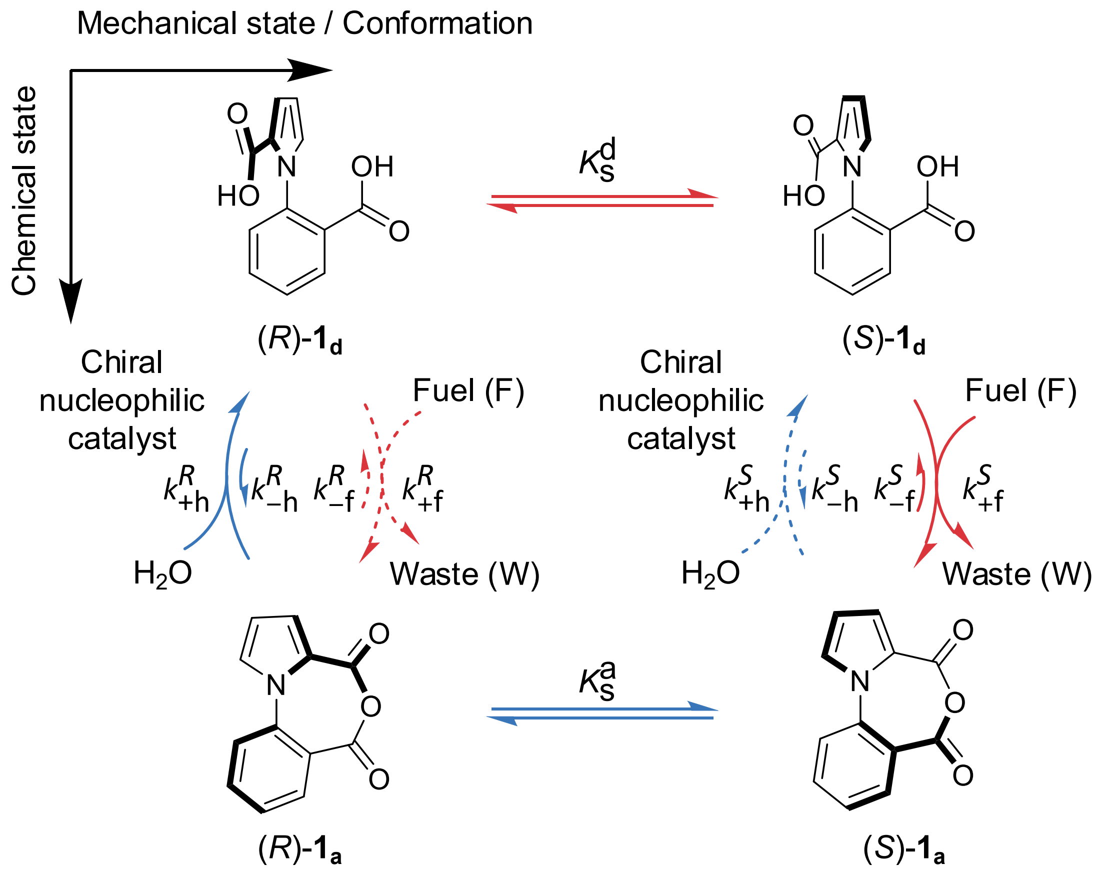
$$K_r = \frac{([F]k^S_{+f} + k^S_{-h})}{([F]k^R_{+f} + k^R_{-h})} \times \frac{([W]k^R_{-f} + [H_2O]k^R_{+h})}{([W]k^S_{-f} + [H_2O]k^S_{+h})} \times K_s^d \times K_s^a$$

$$F_{C-H} = \frac{k^S_{+f}k^R_{+h}}{k^R_{+f}k^S_{+h}} K_s^d K_s^a$$

K_r can be approximated as F_{C-H}

Amano *et al.*, *J. Am. Chem. Soc.* **144**, 20153–20164 (2022)

Stronger Connection between K_r and F_{C-H}



$$K_r = \frac{([F]k_{+f}^S + k_{-h}^S)}{([F]k_{+f}^R + k_{-h}^R)} \times \frac{([W]k_{-f}^R + [H_2O]k_{+h}^R)}{([W]k_{-f}^S + [H_2O]k_{+h}^S)} \times K_s^d \times K_s^a$$

$$= \frac{\gamma + 1 + F_{C-H} \times e^{\Delta\mu/RT}}{\gamma + F_{C-H} + e^{\Delta\mu/RT}} \quad (\Delta\mu = \mu_F + \mu_{H_2O} - \mu_W)$$

$$(numerator) - (denominator)$$

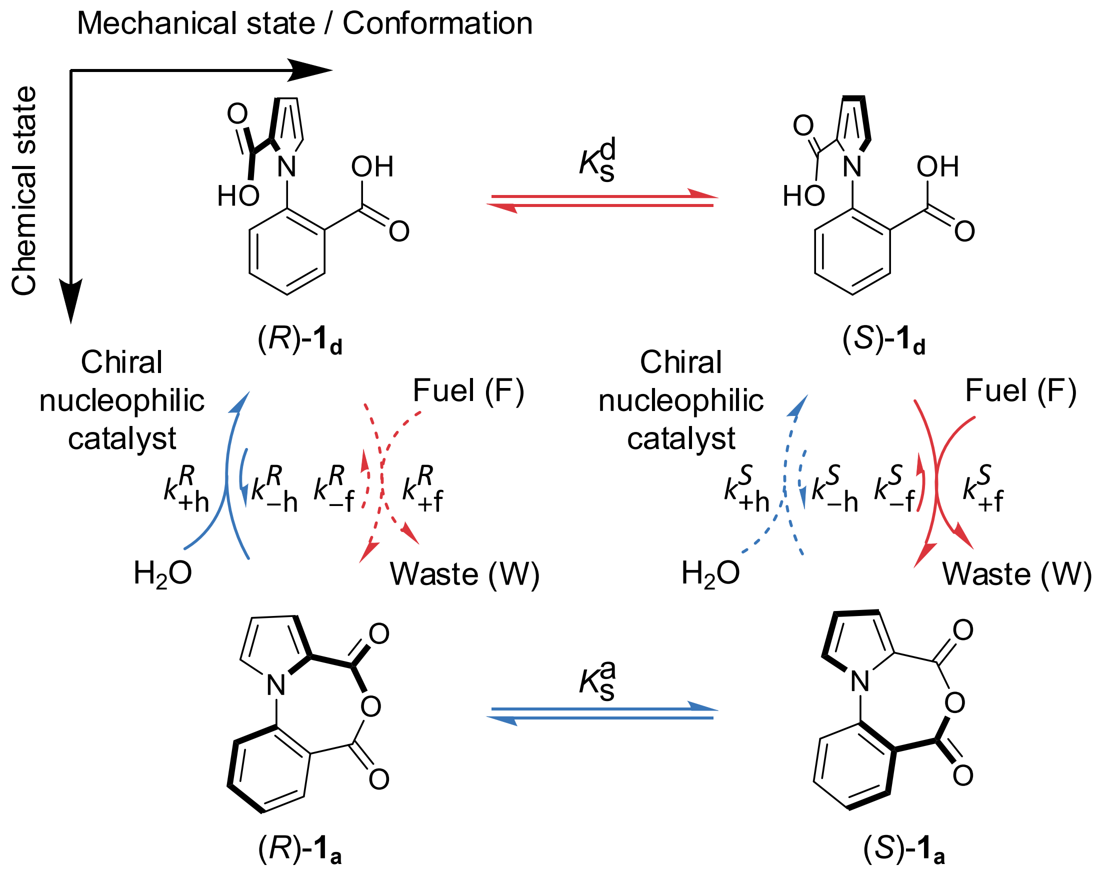
$$= (F_{C-H} - 1) \times (e^{\Delta\mu/RT} - 1)$$

$$\Delta\mu = 0$$

$K_r = 1$ ❌ No directional motion

Amano *et al.*, *J. Am. Chem. Soc.* **144**, 20153–20164 (2022)

Stronger Connection between K_r and F_{C-H}



$$K_r = \frac{([F]k_{+f}^S + k_{-h}^S)}{([F]k_{+f}^R + k_{-h}^R)} \times \frac{([W]k_{-f}^R + [H_2O]k_{+h}^R)}{([W]k_{-f}^S + [H_2O]k_{+h}^S)} \times K_S^d \times K_S^a$$

$$= \frac{\gamma + 1 + F_{C-H} \times e^{\Delta\mu/RT}}{\gamma + F_{C-H} + e^{\Delta\mu/RT}} \quad (\Delta\mu = \mu_F + \mu_{H_2O} - \mu_W)$$

$$(numerator) - (denominator)$$
$$= (F_{C-H} - 1) \times (e^{\Delta\mu/RT} - 1)$$

$\Delta\mu > 0$

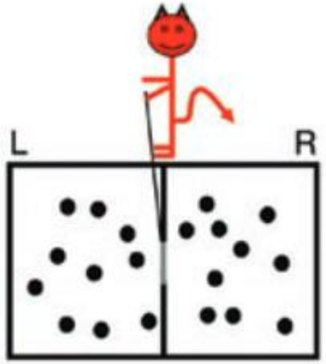
$F_{C-H} > 1$	$K_r > 1$	
$F_{C-H} < 1$	$K_r < 1$	
$F_{C-H} = 1$	$K_r = 1$	❌ No directional motion

Amano *et al.*, *J. Am. Chem. Soc.* **144**, 20153–20164 (2022)

Maxwell’s Demon: Violation of the Second Law of Thermodynamics?

James Clerk Maxwell
1831–1879

High entropy



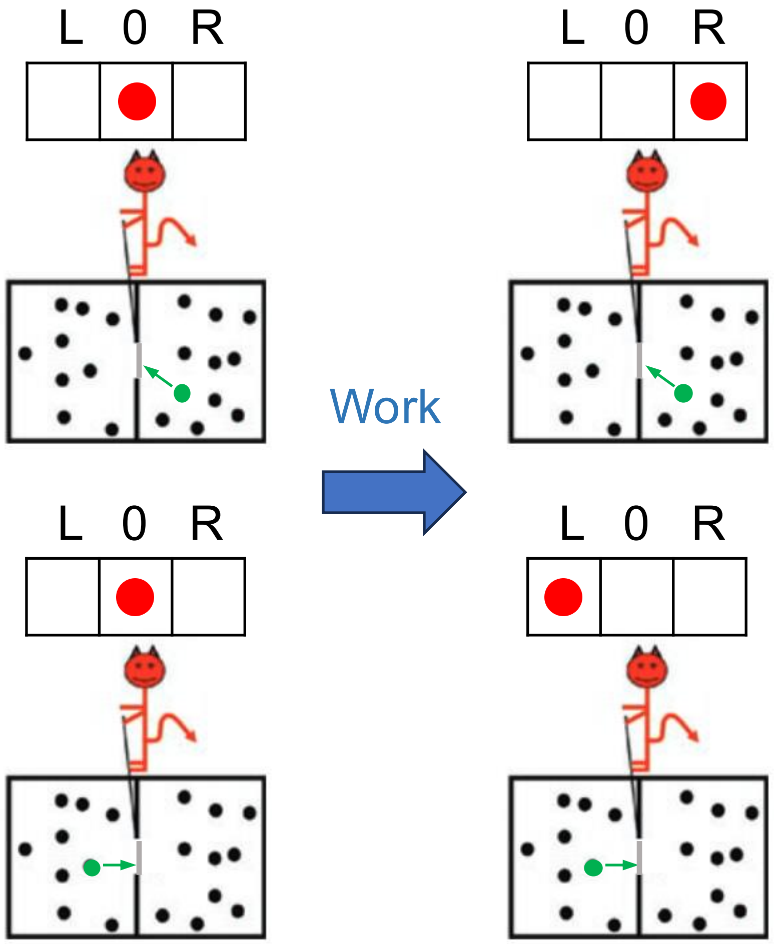
Low entropy

Entropy of the system can be decreased without performing work?

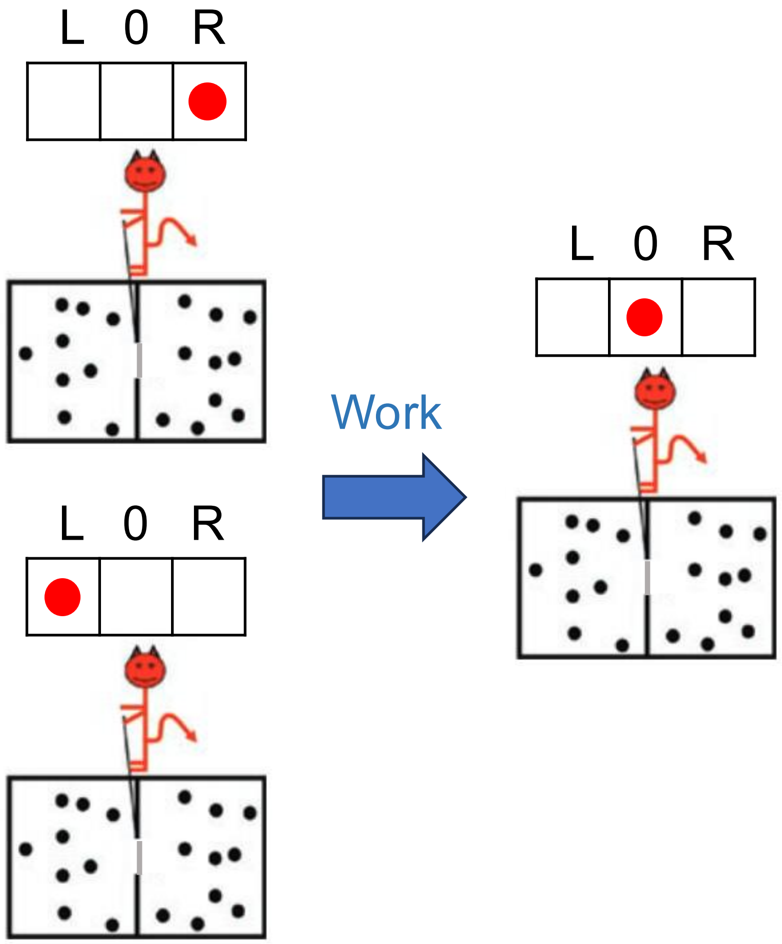
Kay *et al.*, *Angew. Chem. Int. Ed.* **46**, 72–191 (2007)

Resolution: Energetic Cost of Information Processing

- Measurement

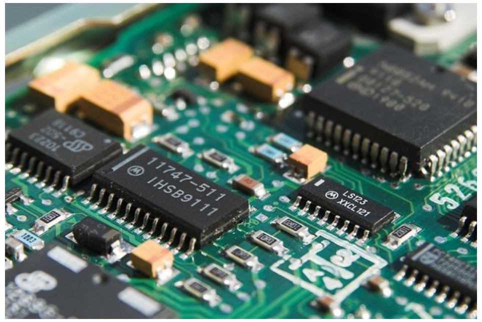


- Memory erasure



Sagawa, *Thermodynamics of Information Processing* (2014)

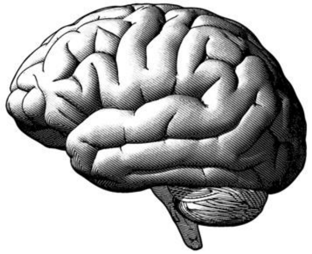
Advent of Information Thermodynamics



- Attempts to resolve the paradox of Maxwell's demon

- "Information is physical." R. Landauer, *Phys. Today* **44**, 23−29 (1991).

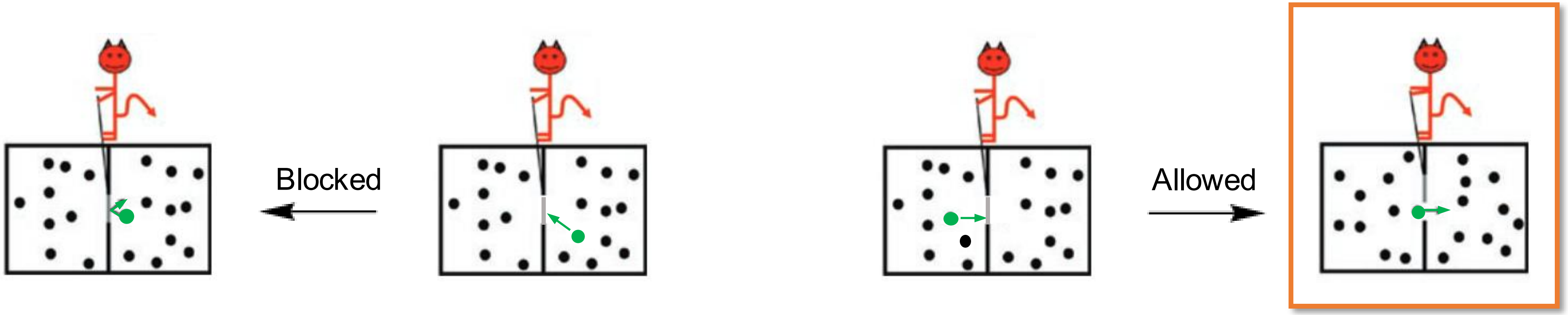
- Connection between entropy and quantity of information



$$S = k_B log W \qquad I(E) = -log P(E)$$

Parrondo *et al.*, *Nat. Phys.* **11**, 131−139 (2015).

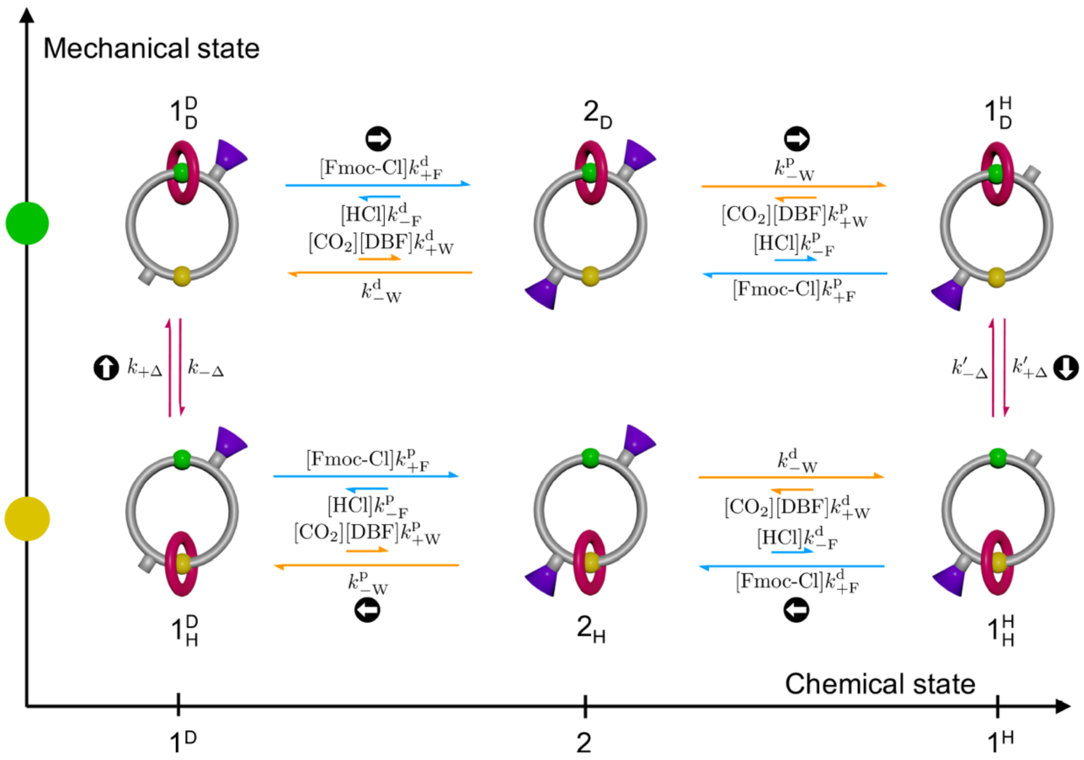
Similarity of Maxwell’s Demon and Brownian Ratchets

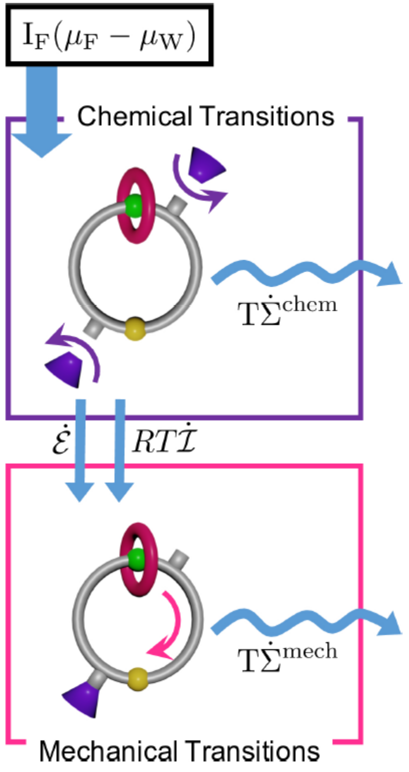


Can information thermodynamics be applied to analysis of Brownian ratchets?

Kay *et al.*, *Angew. Chem. Int. Ed.* **46**, 72–191 (2007)　　Amano *et al.*, *Nature* **594**, 529–534 (2021)

Energy Transduction to Mechanical Transitions Drives Directional Motion





$$\dot{\mathcal{E}} = J(\mu^\circ_{1^D_H} - \mu^\circ_{1^D_D} + \mu^\circ_{1^H_D} - \mu^\circ_{1^H_H})$$

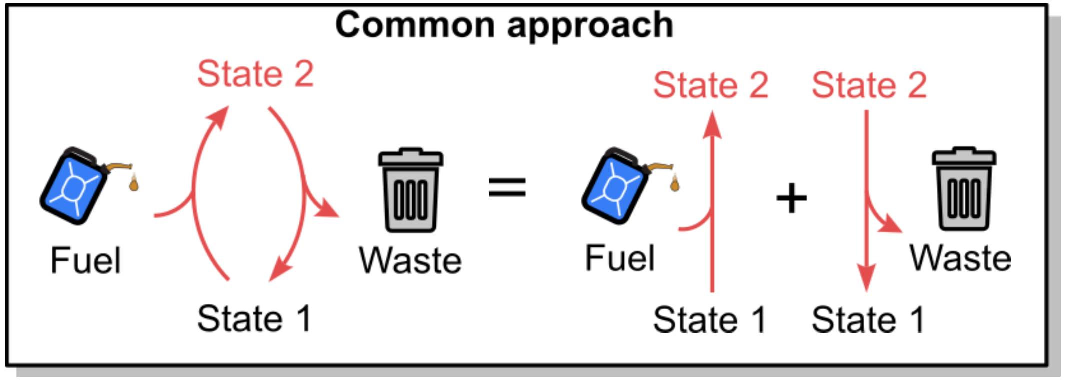
Energy flow

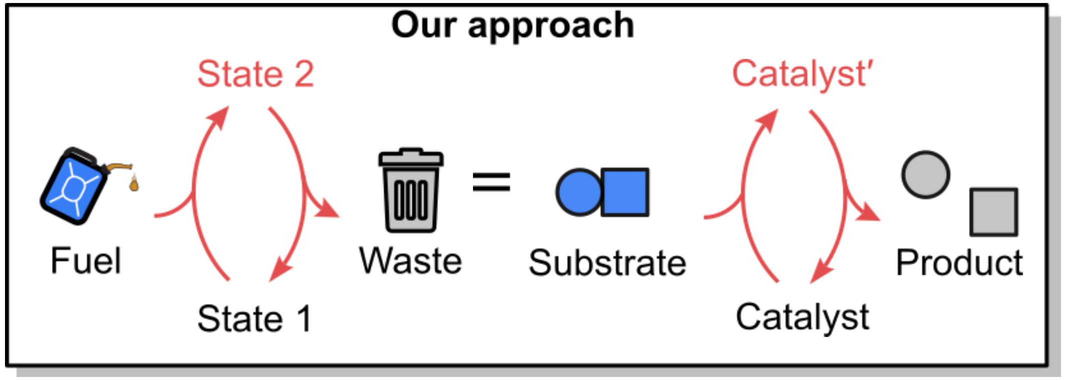
$$RT\dot{\mathcal{I}} = JRT \log \frac{[1^D_H][1^H_D]}{[1^D_D][1^H_H]}$$

Information flow

Amano *et al.*, *Nat. Chem.* **14**, 530−537 (2022)

Simple and General Approach for Developing a Chemical Reaction Cycle





❌ ***Difficult to find compatible reactions***

❌ ***Background fuel decomposition***

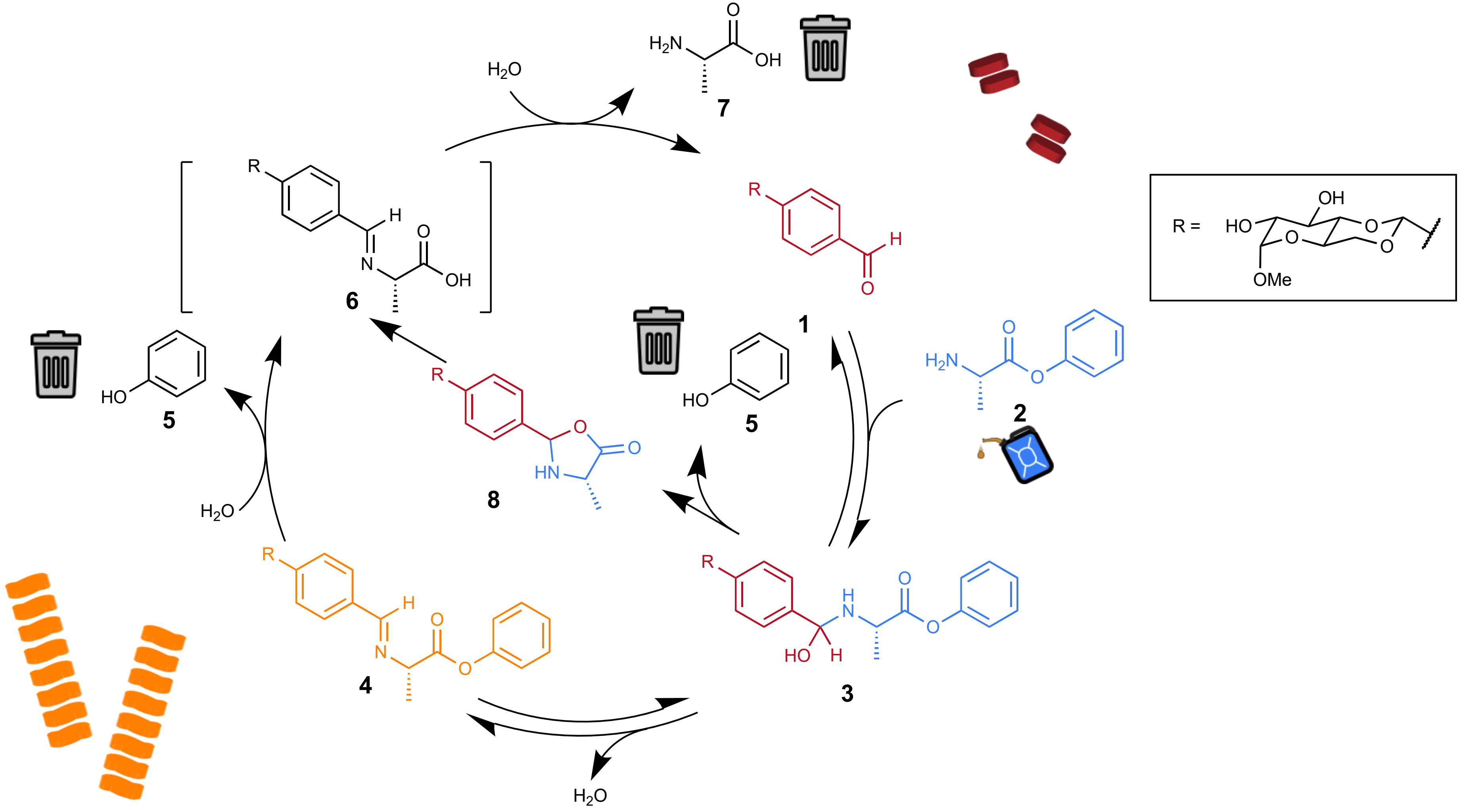
✓ ***Compatibility of multiple processes***

✓ ***Suppress background fuel decomposition***

Amano *et al.*, *Nat. Nanotechnol.* **16**, 1057–1067 (2021)

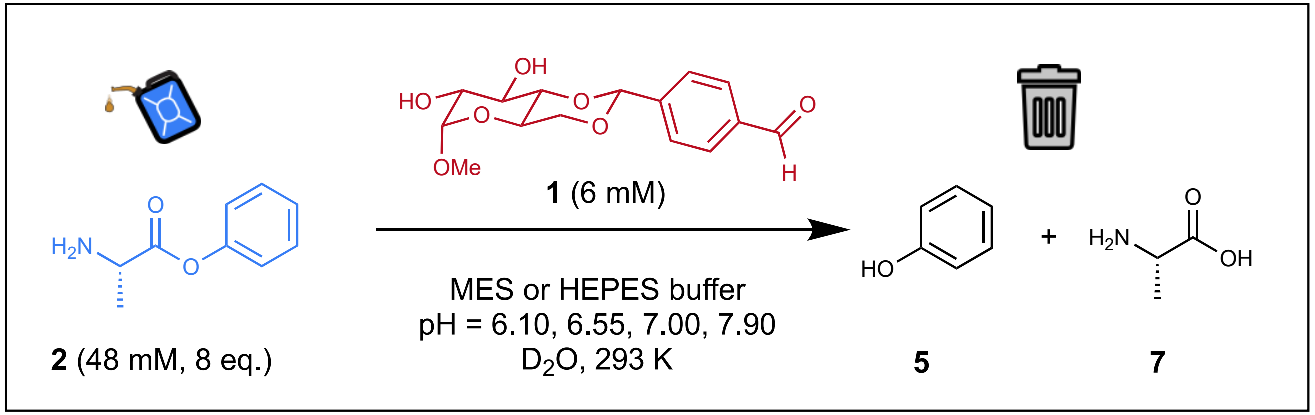
Amano, Hermans, *J. Am. Chem. Soc.* **146**, 23289–23296 (2024)

Aim: Transient Self-Assembly

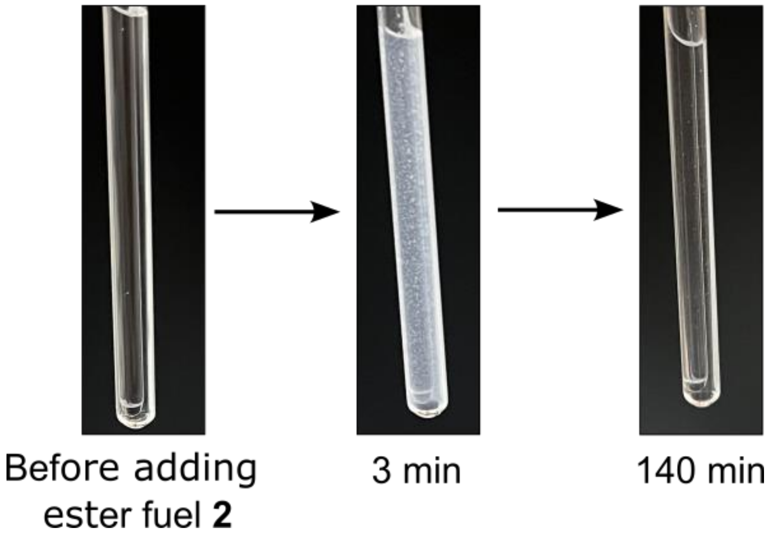


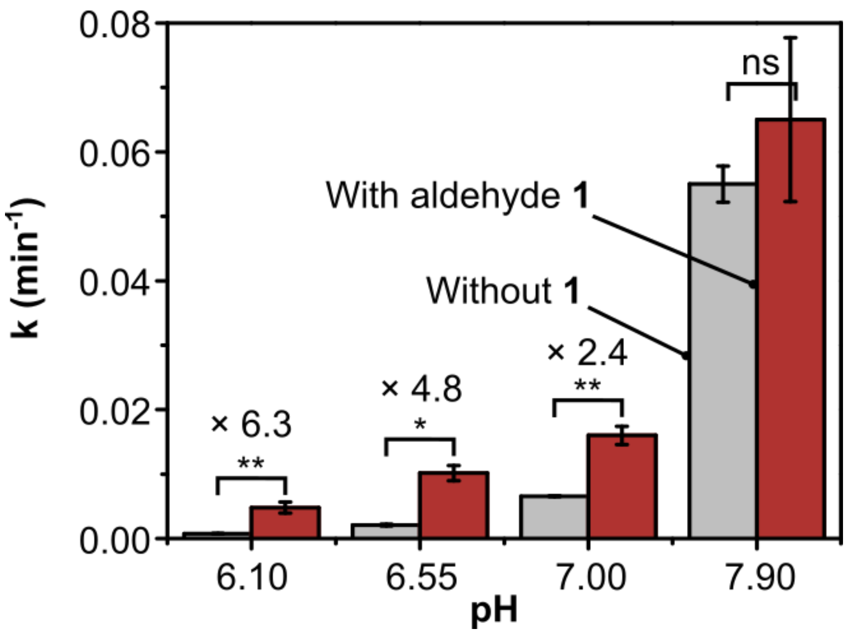
Amano, Hermans, *J. Am. Chem. Soc.* **146**, 23289–23296 (2024)

Observation of Transient Self-Assembly



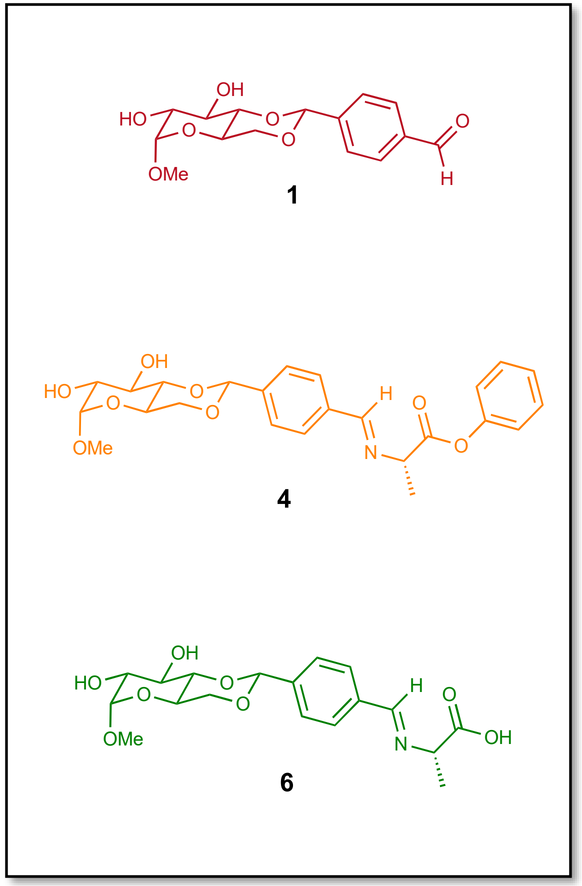
pH 7.90

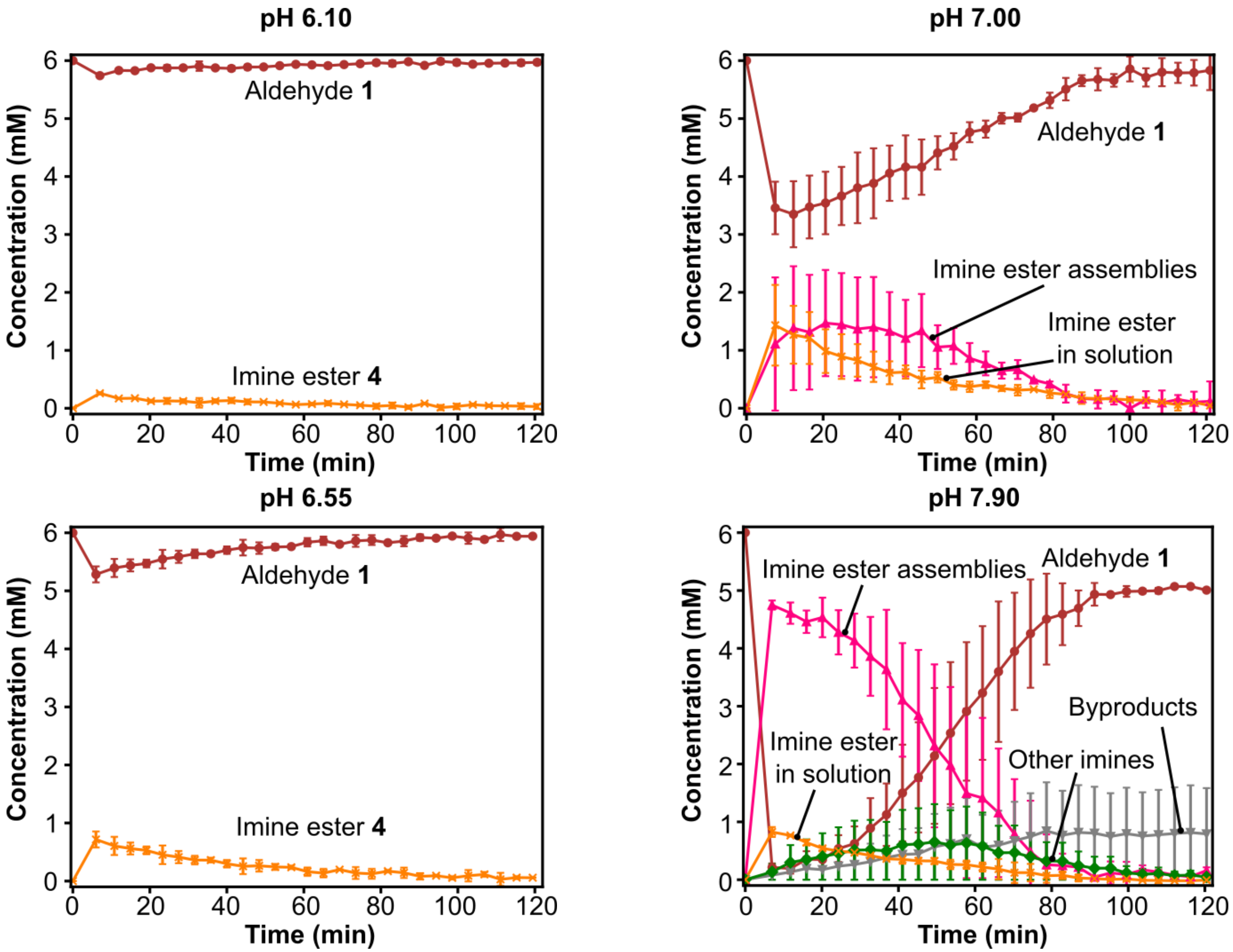




Amano, Hermans, *J. Am. Chem. Soc.* **146**, 23289–23296 (2024)

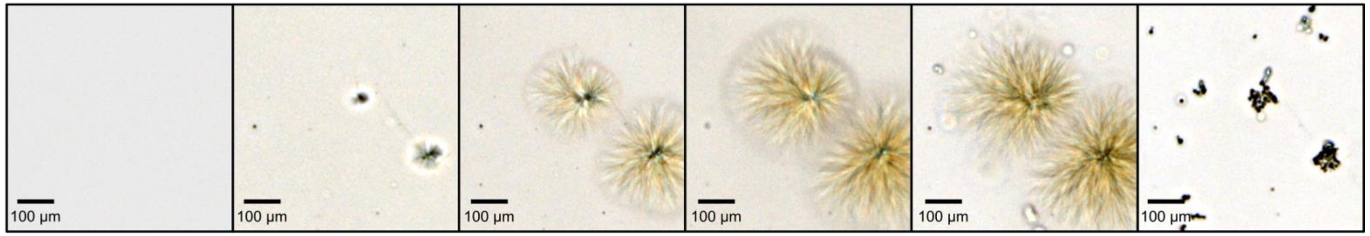
Effect of pH to Imine Ester 4 Formation





Higher pH, more imine ester 4

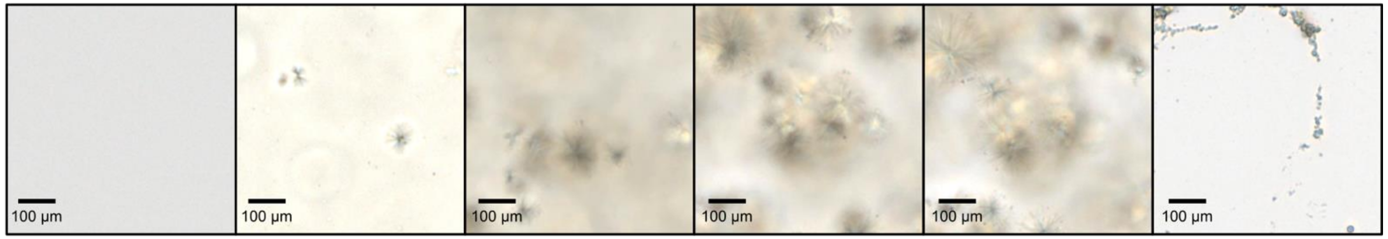
Observation by Optical Microscope – pH 7.00



pH 7.00 Before fuel addition 4 min 5 min 6 min 60 min 86 min

Amano, Hermans, *J. Am. Chem. Soc*. **146**, 23289–23296 (2024)

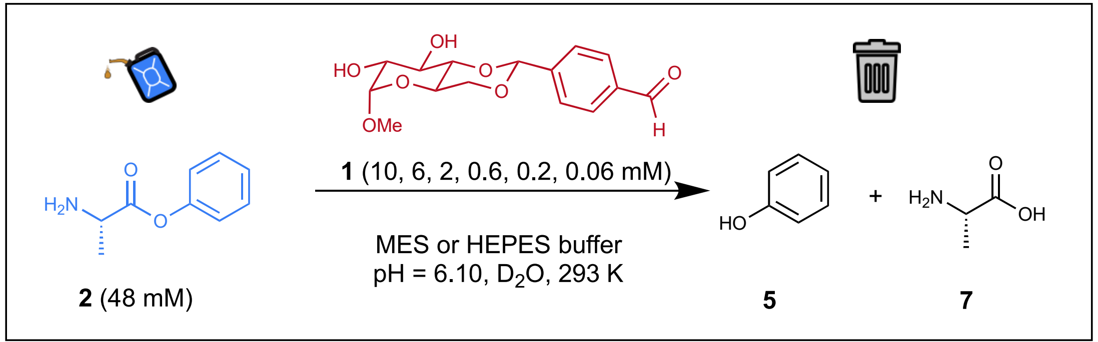
Observation by Optical Microscope – pH 7.90



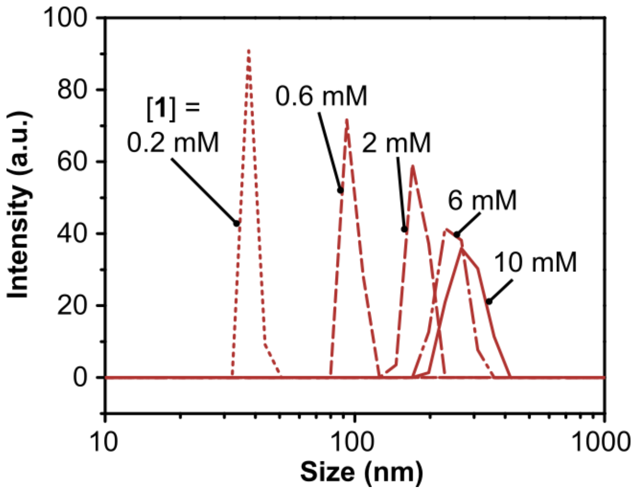
pH 7.90 Before fuel addition 1 min 8 min 15 min 30 min 65 min

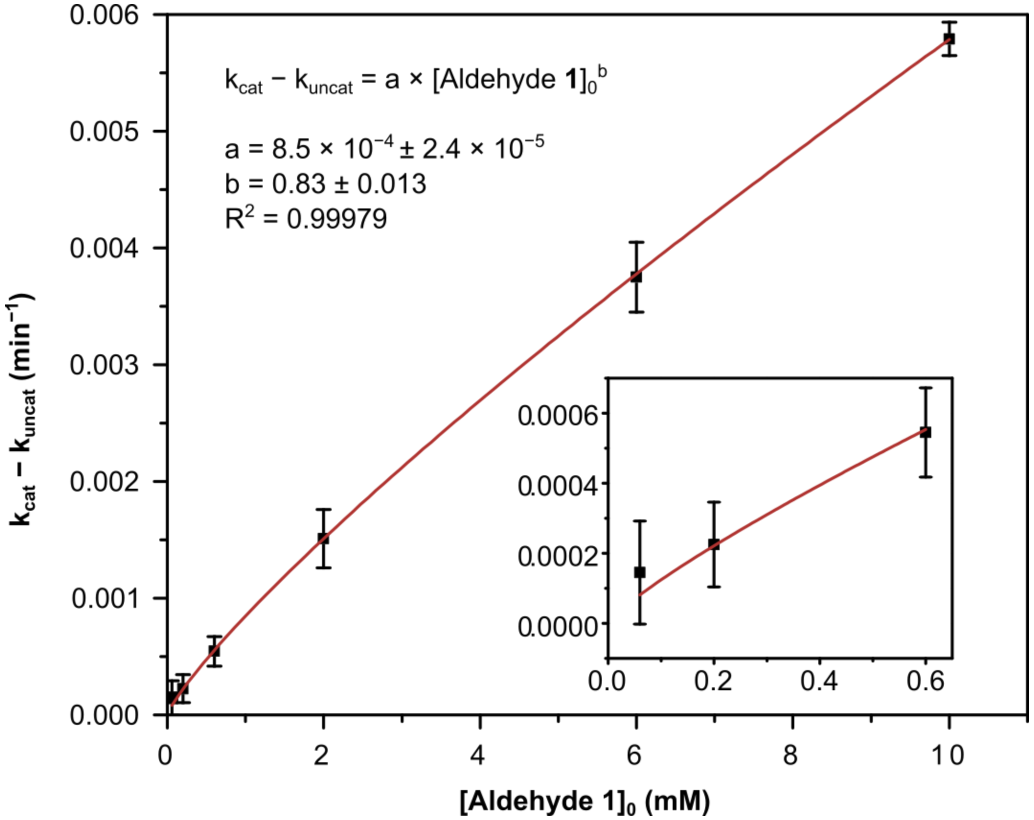
Amano, Hermans, *J. Am. Chem. Soc.* **146**, 23289–23296 (2024)

Can Assemblies of Aldehyde 1 Catalyze Fuel Decomposition?



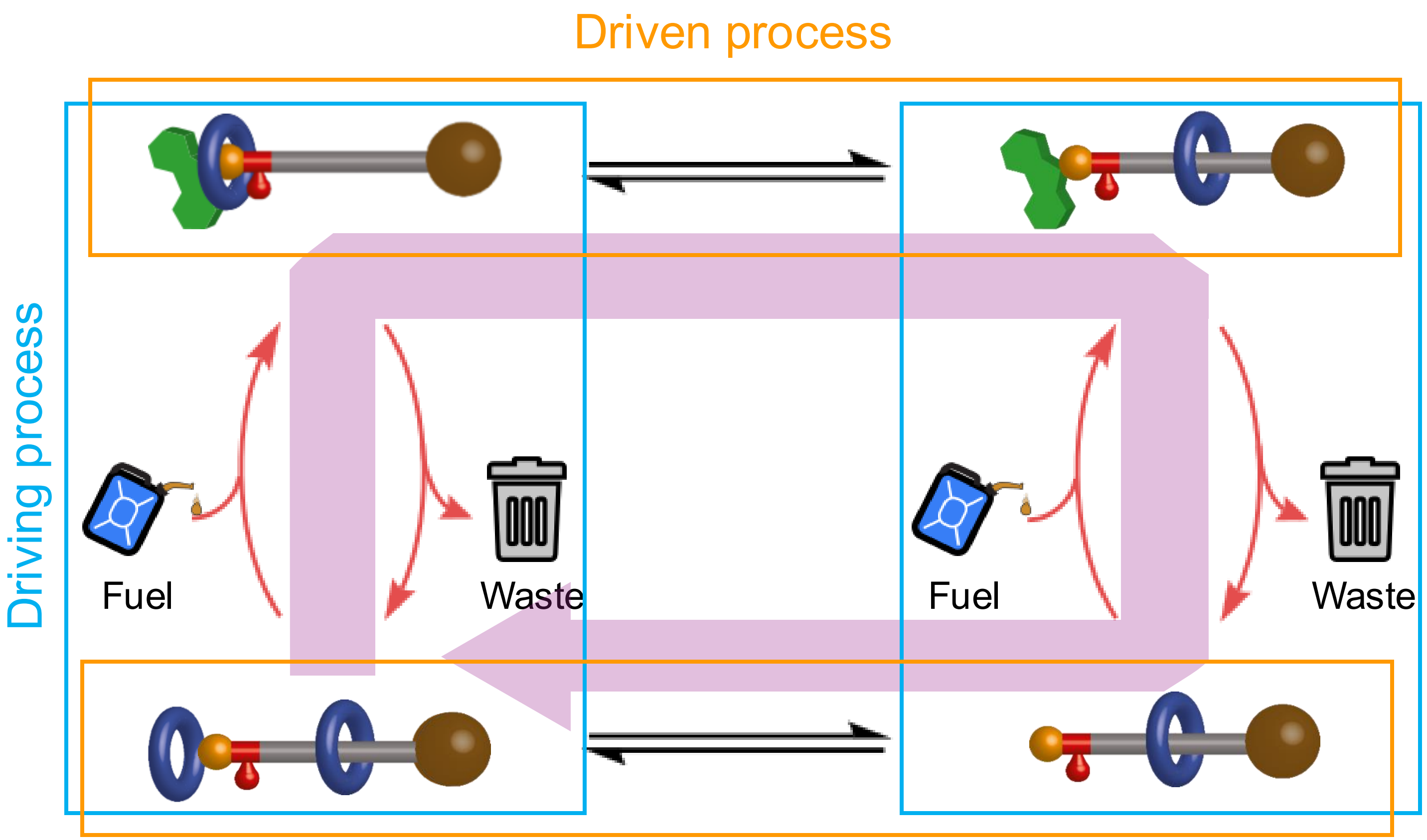
Dynamic Light Scattering





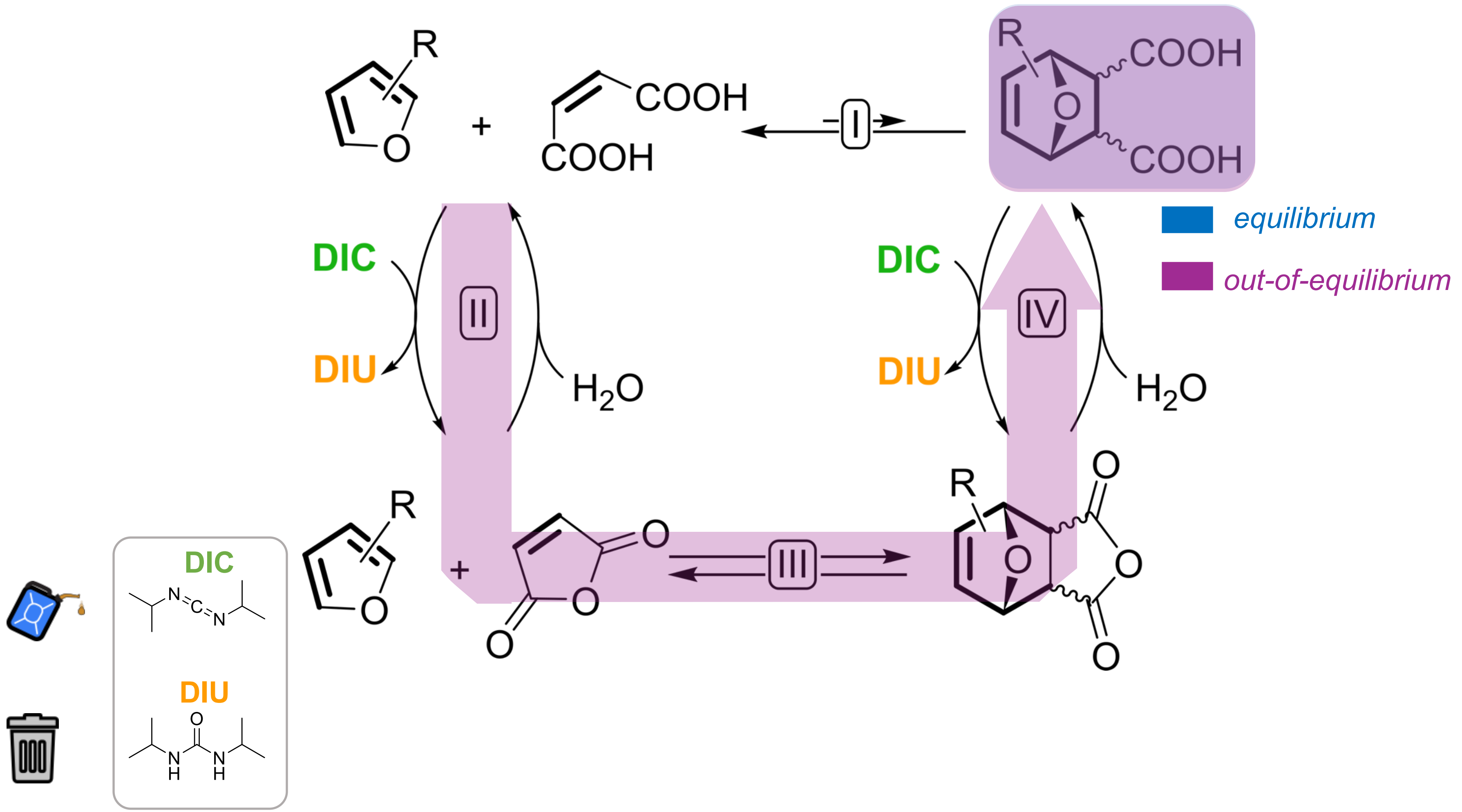
Assemblies of 1 also catalyse fuel decomposition

Application of Ratchet Mechanism to Other Systems



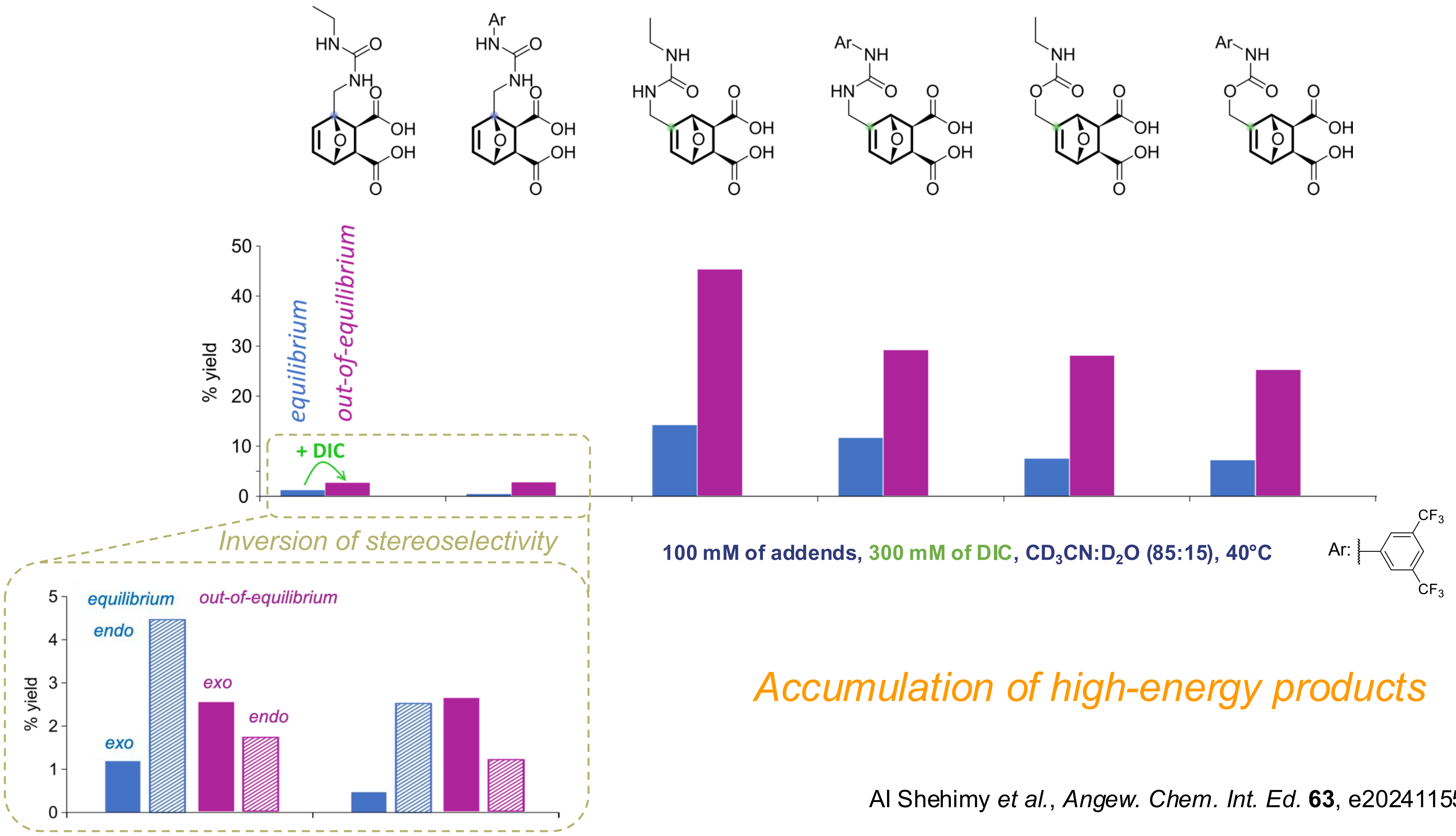
Can ratchet mechanisms drive other processes?

Endergonic Synthesis: Synthesis away from Equilibrium



Al Shehimy *et al.*, *Angew. Chem. Int. Ed.* **63**, e202411554 (2024)

Single-Batch Fuel Addition

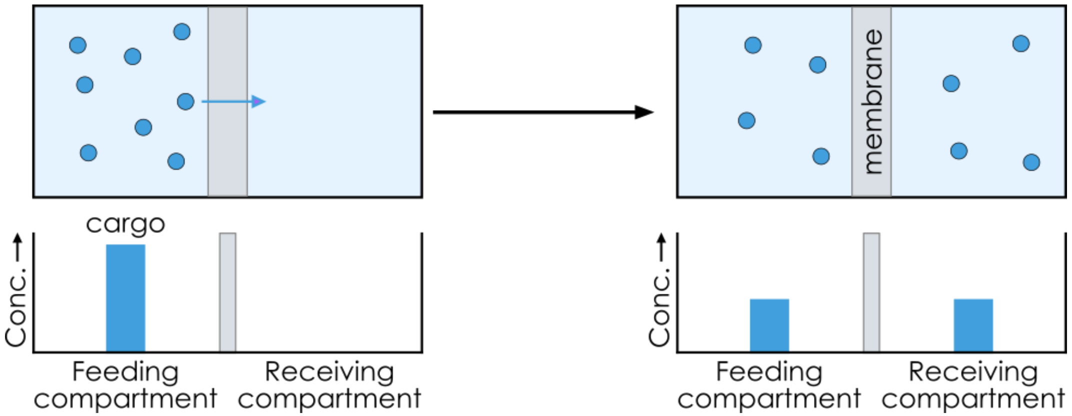


Accumulation of high-energy products

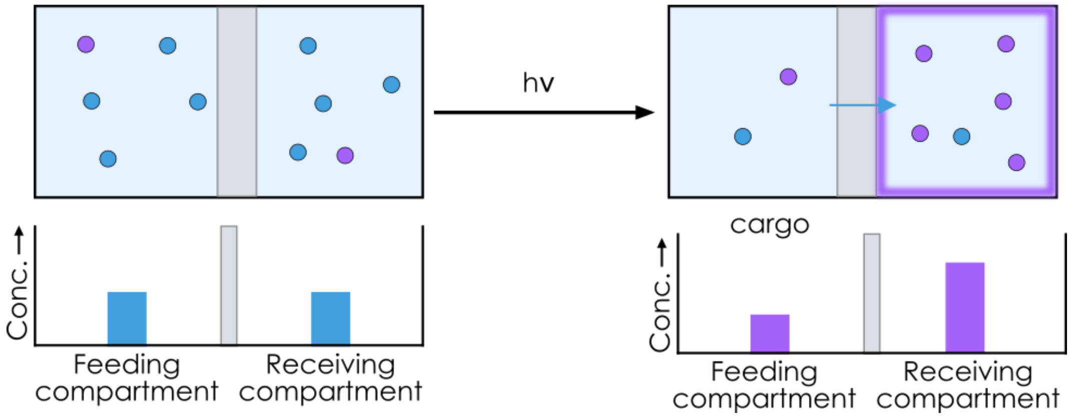
Al Shehimy *et al.*, *Angew. Chem. Int. Ed.* **63**, e202411554 (2024)

Active Transport: Transport against a Concentration Gradient

➢ Passive transport



➢ Active transport

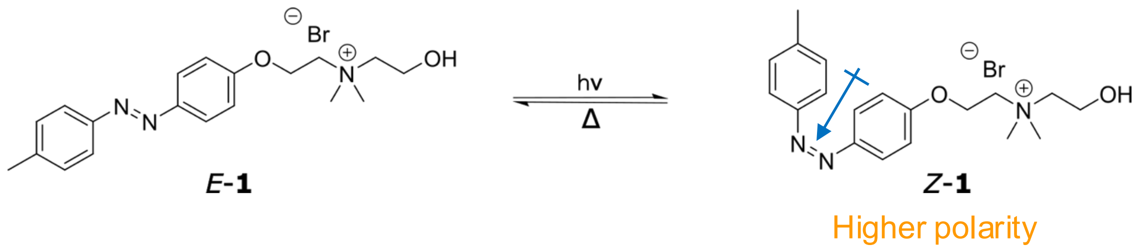


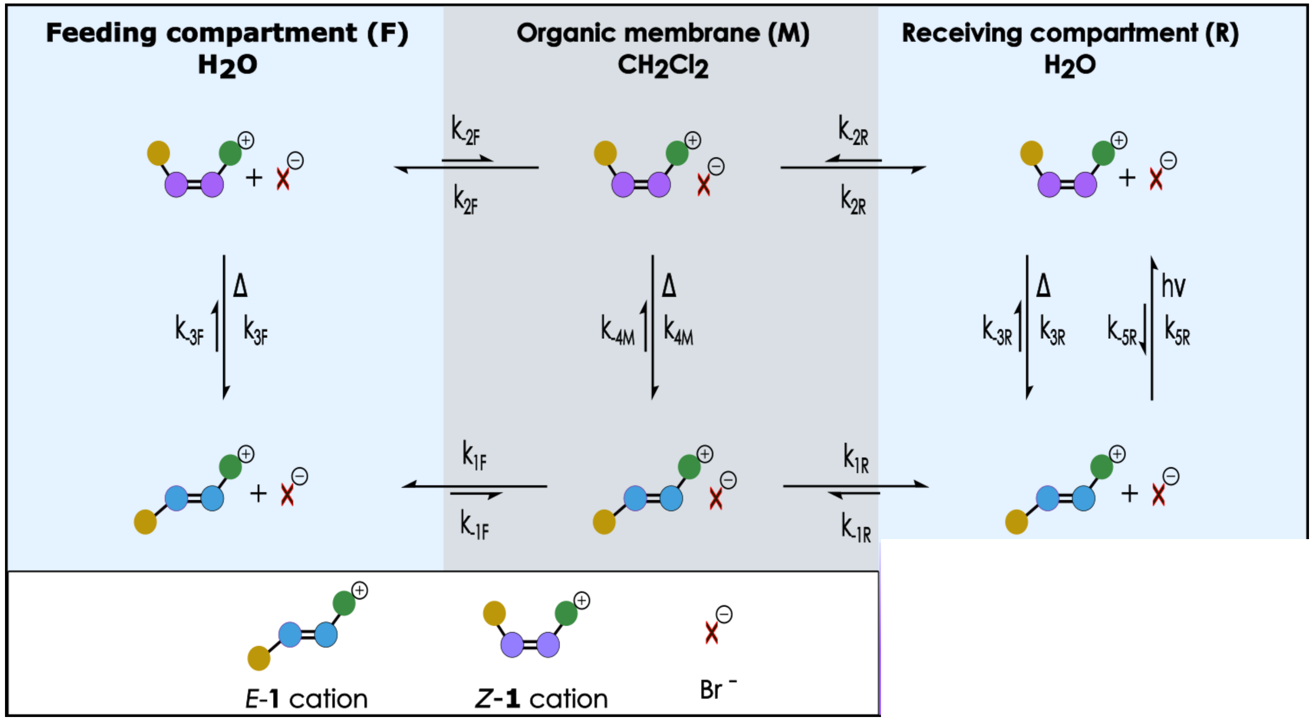
➢ Significant role in biology (e.g., energy conversion, signaling)

➢ Key requirement: supply of energy

Yahaya, Amano *et al.*, *in preparation*

Azobenzene Photoisomerization Coupled to Mass Transport

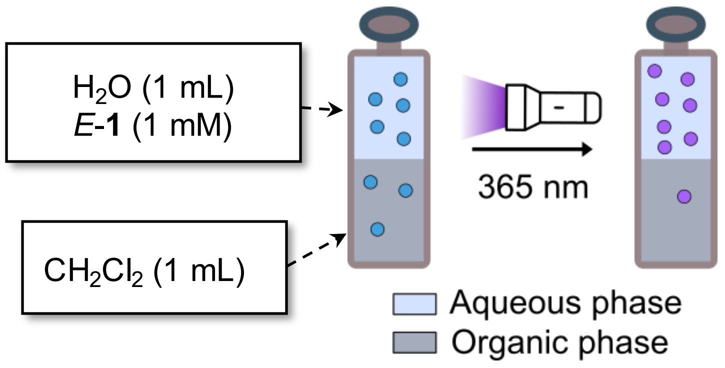




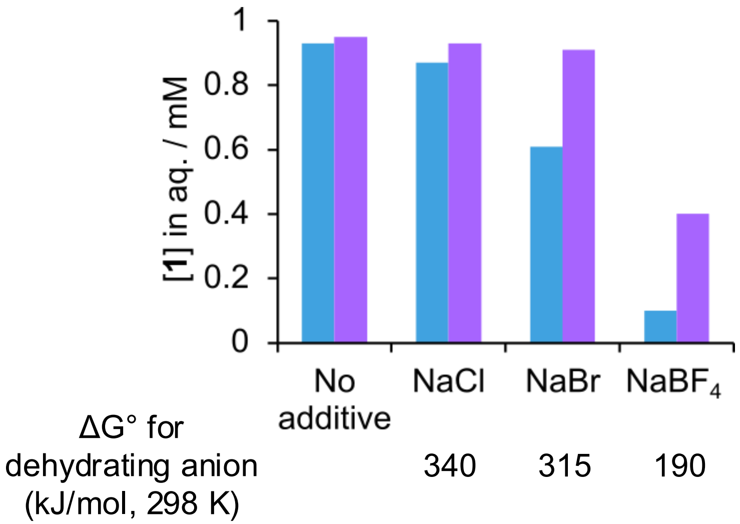
Yahaya, Amano *et al.*, *in preparation*

Repartition Study in Biphasic Systems

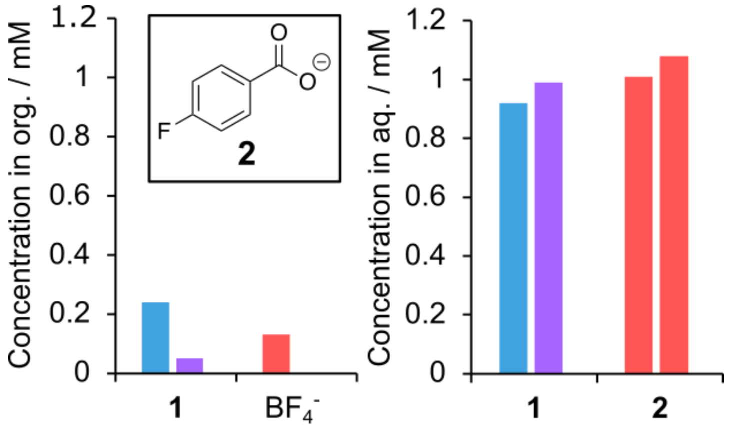
- Setup



- Effect of additives (100 mM)



- Co-transport of **1** and counter anions



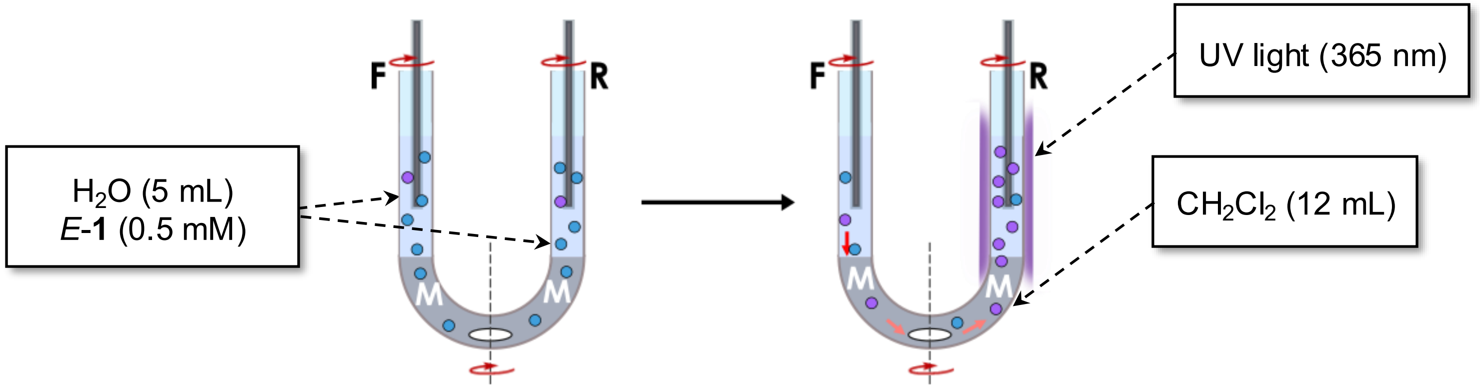
*Additive affects distribution of azobenzene **1***

***1** and added anions are transported together*

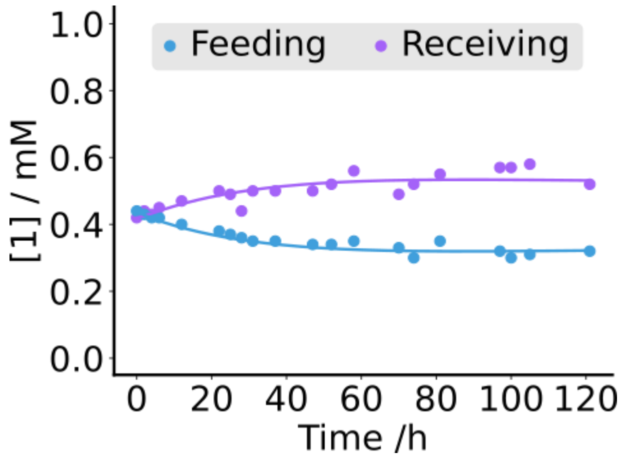
Yahaya, Amano *et al.*, *in preparation*

Active Transport of Azobenzene Derivatives

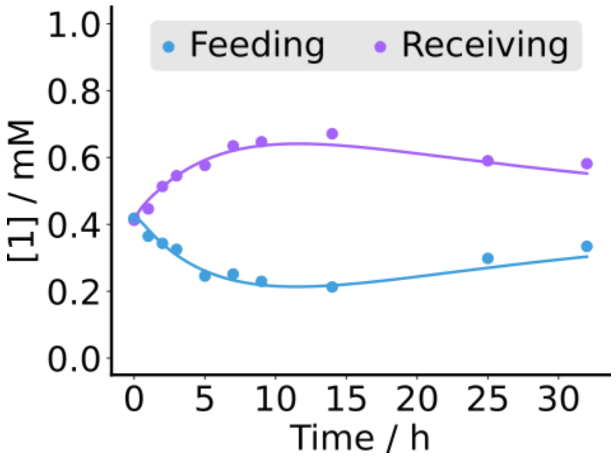
➢ Setup: U-Tube



➢ No additive



➢ With 10 mM NaBr in F and R



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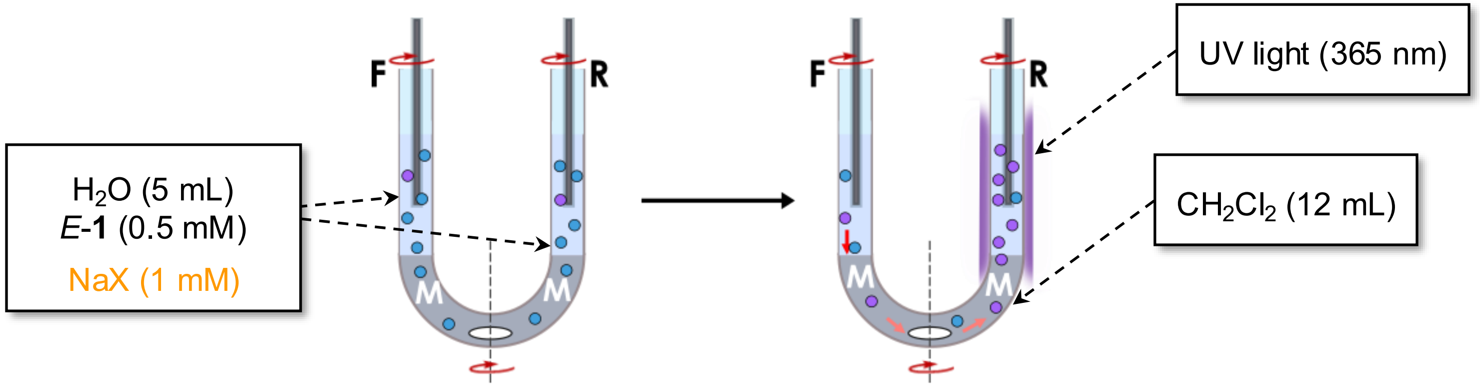
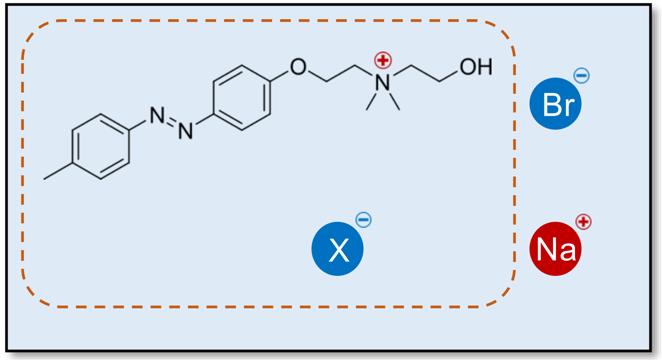
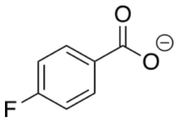
Co-transport of Counter Anions

- Addition of salts

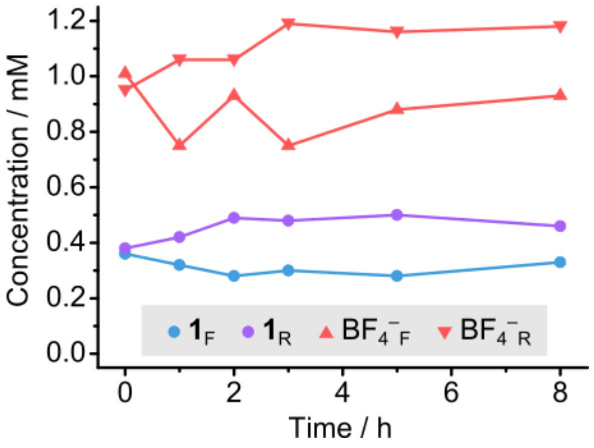


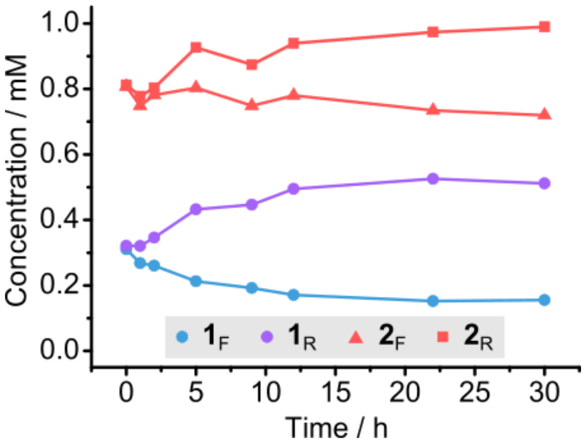


- $X^- = BF_4^-$
- X^- = 4-fluorobenzoate



2



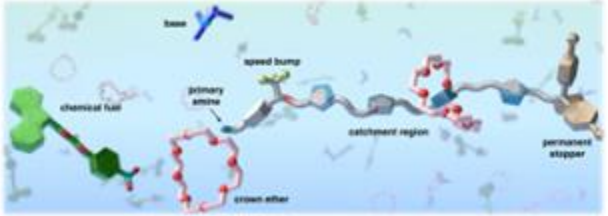


Simple & versatile strategy for active transport of ions

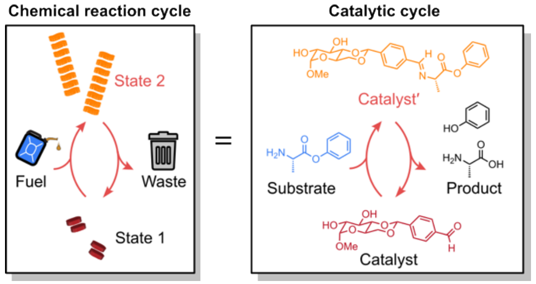
Yahaya, Amano *et al.*, *in preparation*

Conclusion and Outlook

- Autonomous molecular pump

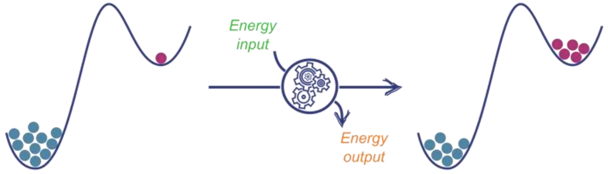


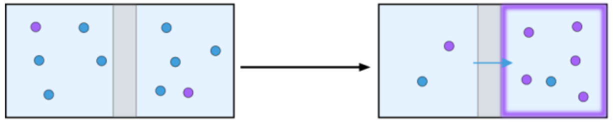
- Simple & general approach for developing chemically driven systems



- Theories for design

- Endergonic synthesis, active transport





Practical systems?

Other applications?

Acknowledgements

- Prof. David Leigh
- Dr. Stephen Fielden
- Dr. Stefan Borsley, Dr. Zhanhu Sun
- Dr. Benjamin Roberts,
  Dr. Elisabeth Kreidt,
  Dr. Emanuele Penocchio,
  Prof. Massimiliano Esposito
- Prof. Thomas Hermans
- Hermans Lab
- Dr. Giulio Ragazzon
- Dr. Shaymaa Al Shehimy,
  Hai Dang Le
- Dr. Federico Nicoli
  Sani Yahaya
- Ragazzon Lab

@ShuntaroAmano

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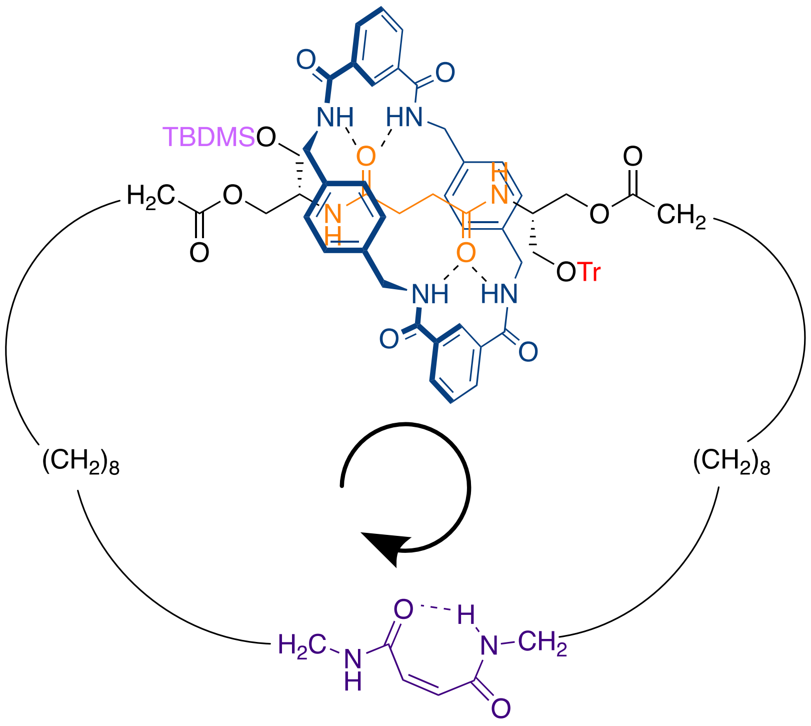
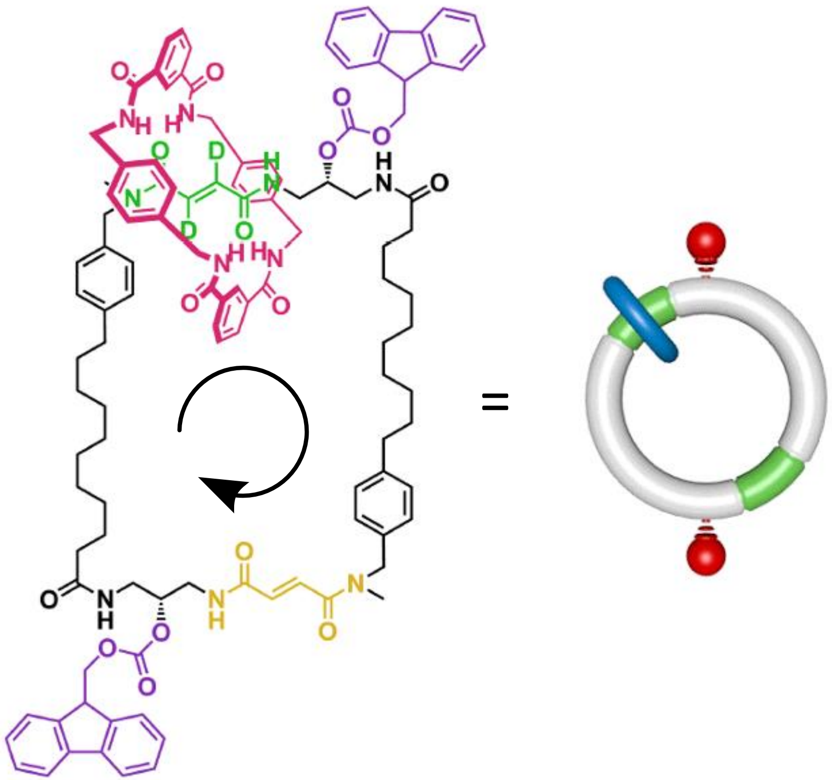
Non-autonomous vs. Autonomous

- ➢ Non-autonomous motor



Stepwise operation

- ➢ Autonomous motor

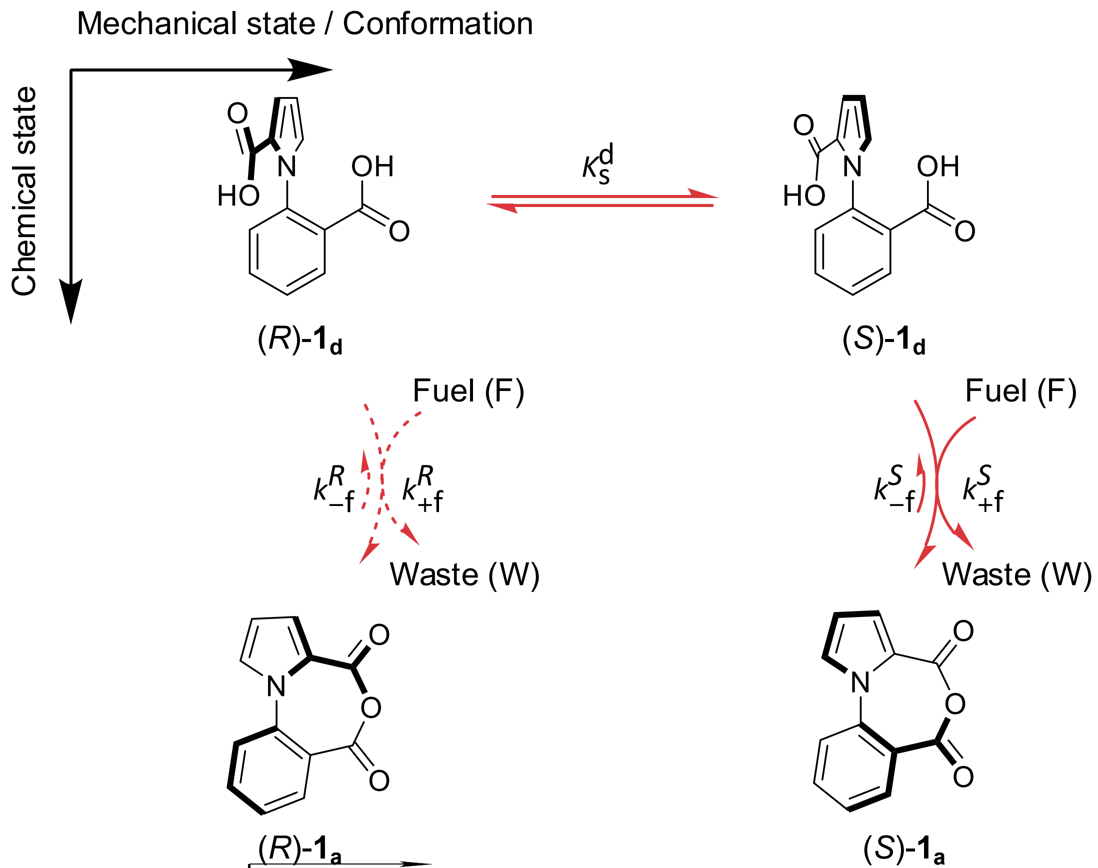


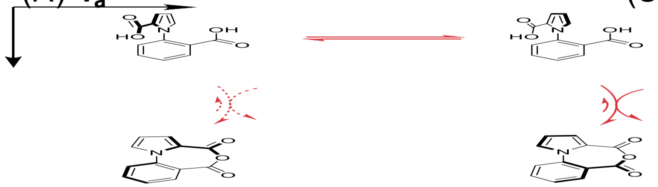
Operation in fixed environments

J. V. Hernández, E. R. Kay & D. A. Leigh *Science* **306**, 1532–1537 (2004).

M. R. Wilson, J. Solà, A. Carlone, S. M. Goldup, N. Lebrasseur, D. A. Leigh, *Nature* **534,** 235-240 (2016).

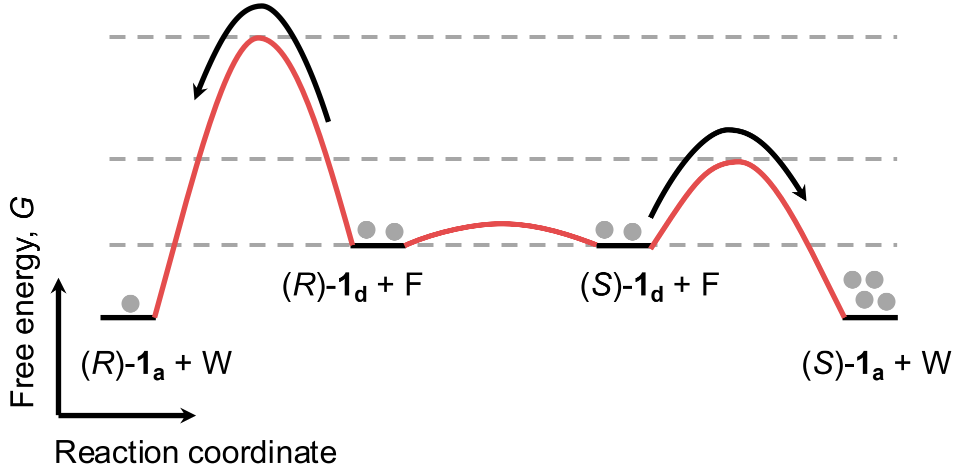
Anhydride Formation





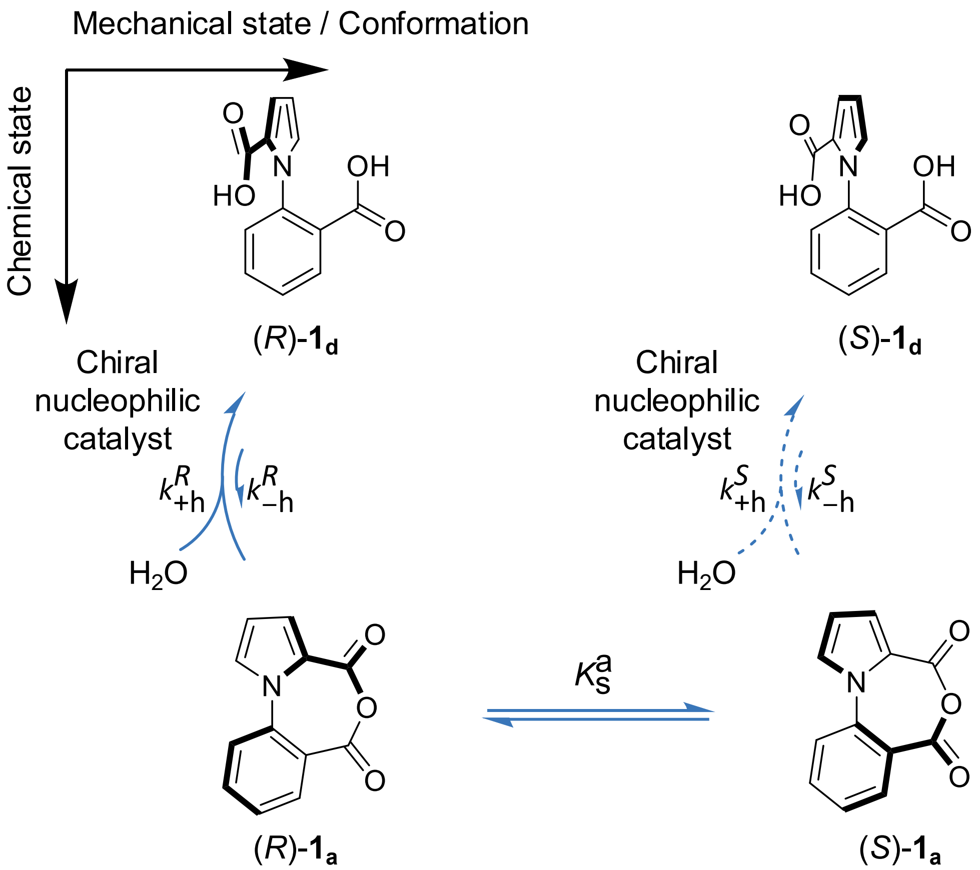
Carbodiimide fuel (F)

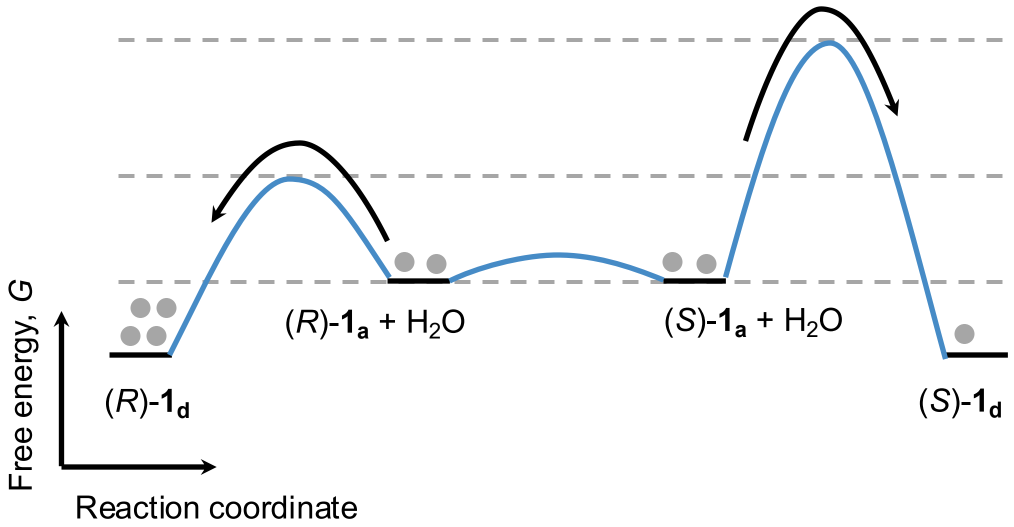
Urea waste (W)



$$\frac{[(S)-\mathbf{1}_a]}{[(R)-\mathbf{1}_a]} \approx \frac{k^S_{+f}}{k^R_{+f}} K^d_s$$

Hydrolysis

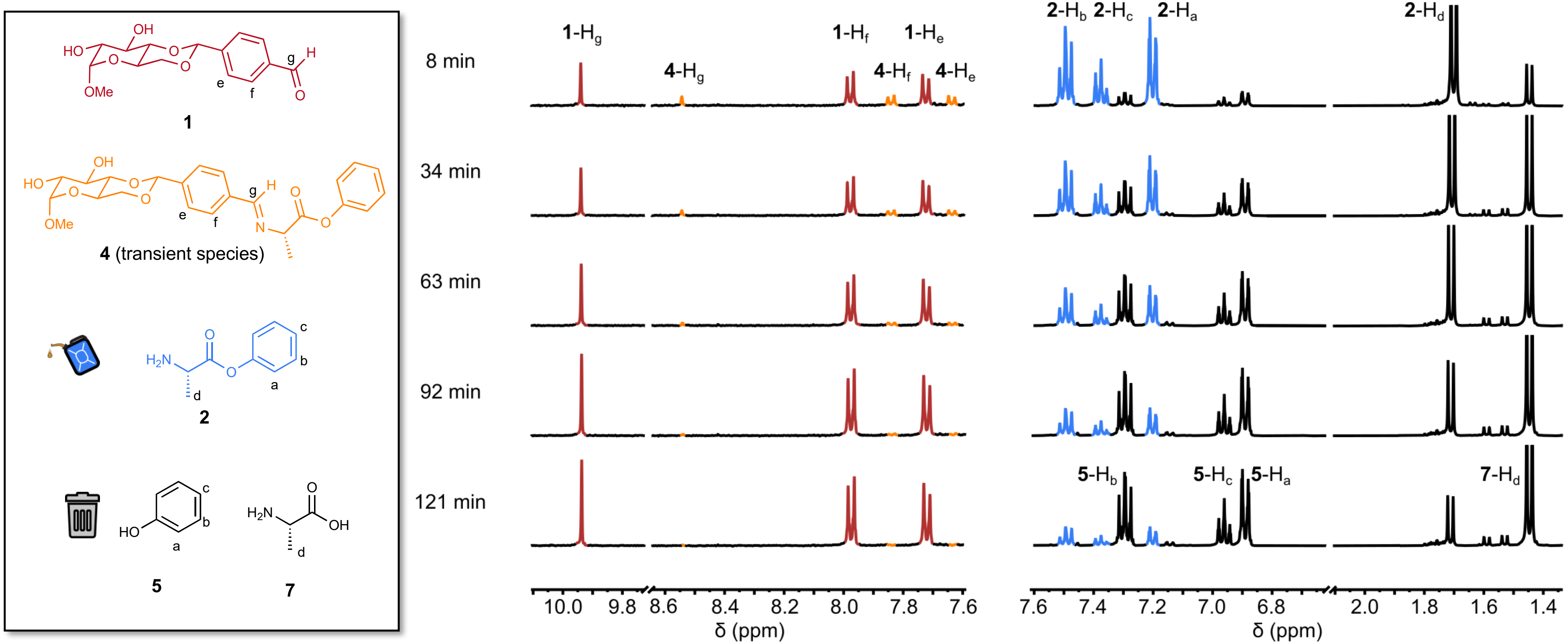




$$\frac{[(R)-\mathbf{1}_d]}{[(S)-\mathbf{1}_d]} \approx \frac{k^R_{+h}}{k^S_{+h}} K^a_s$$

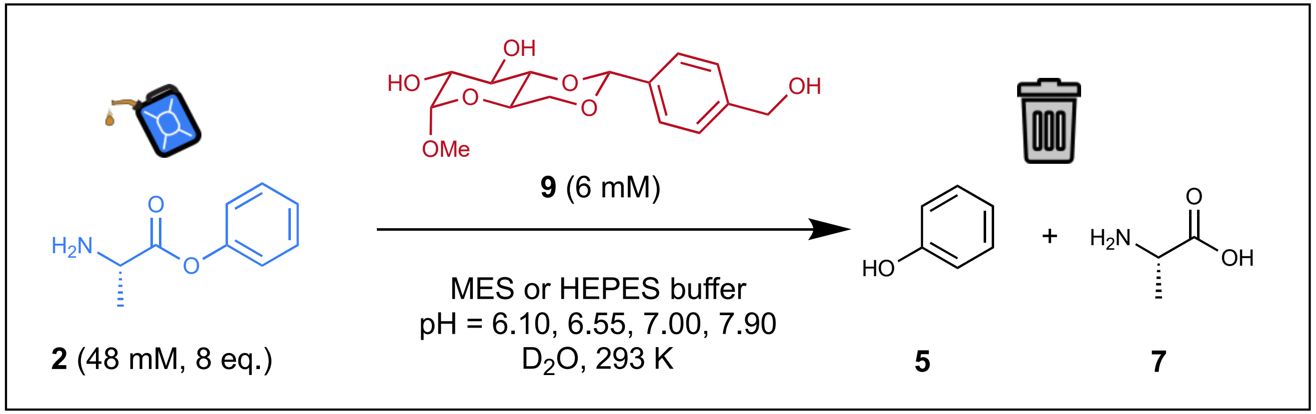
Transient Formation of Imine Ester 4

^{1}H NMR in D_2O (pH 7.00)

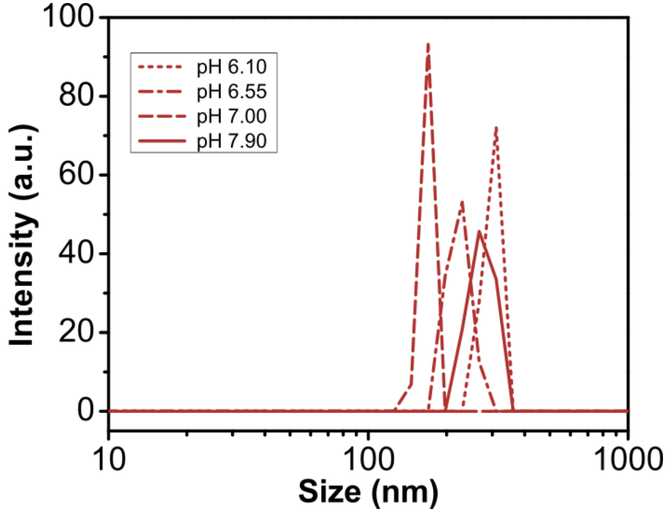


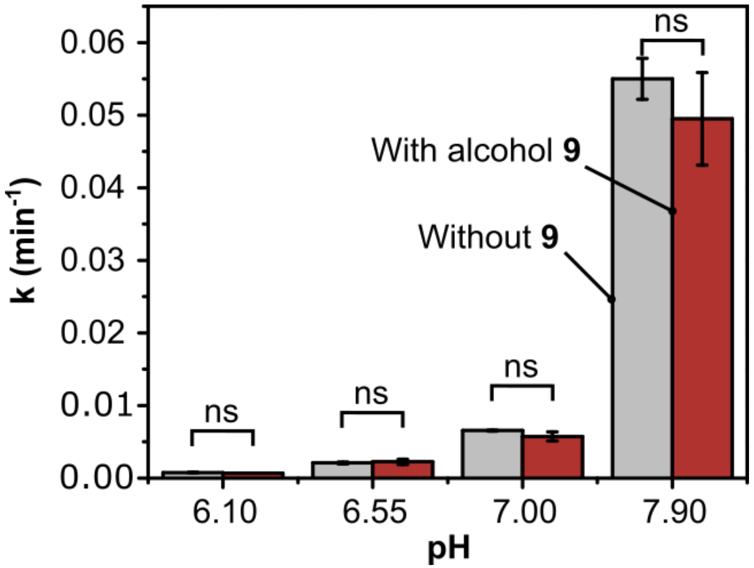
Amano, Hermans, *J. Am. Chem. Soc.* **146**, 23289–23296 (2024)

Control Experiment with Alcohol 9



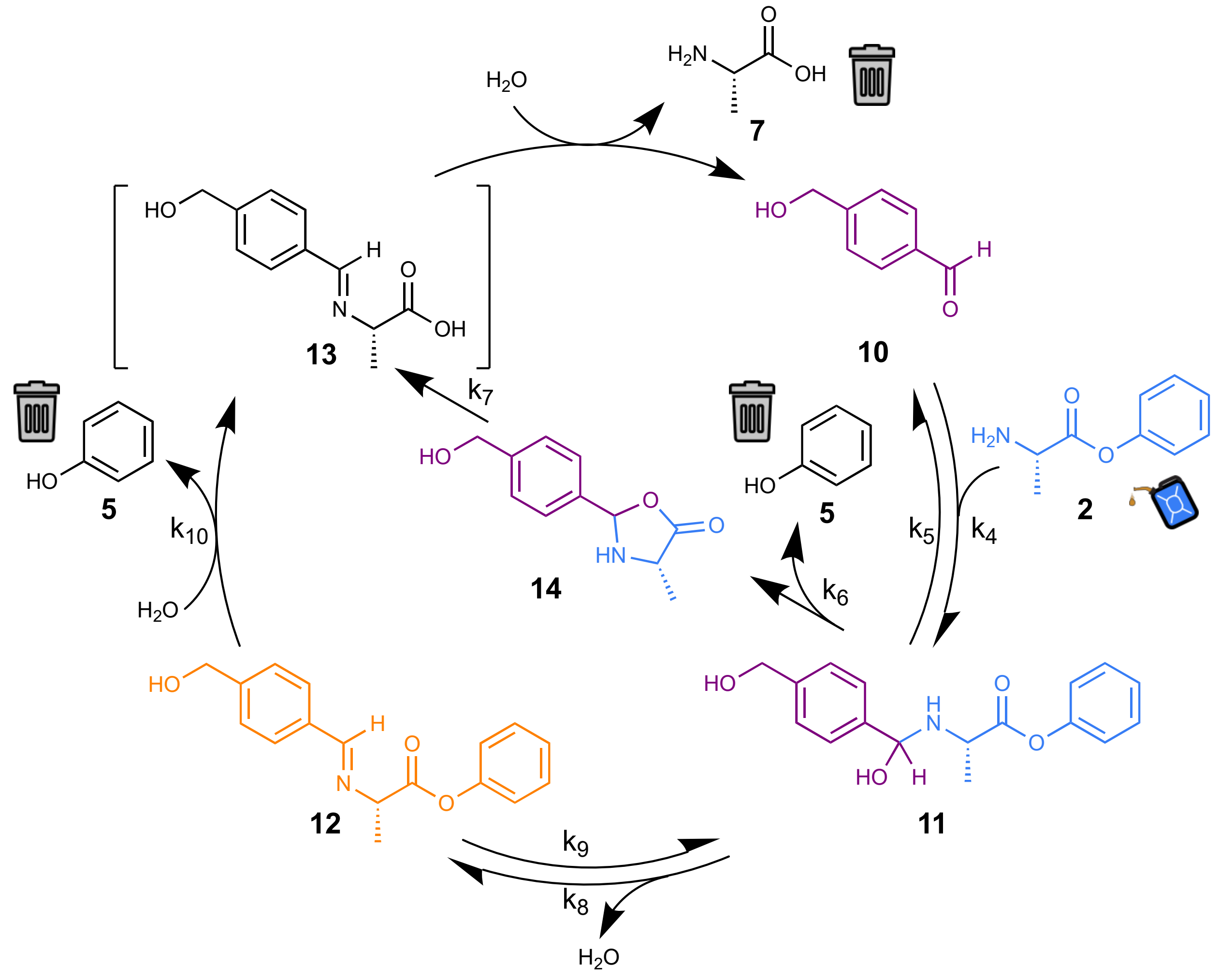
Dynamic Light Scattering



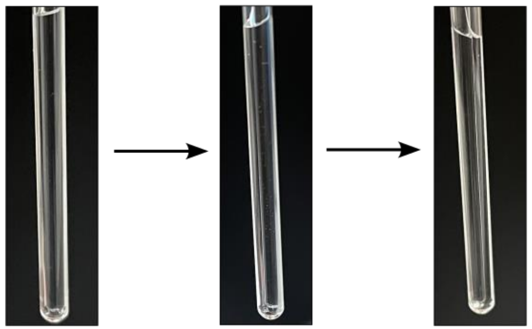


No aldehyde, no catalysis

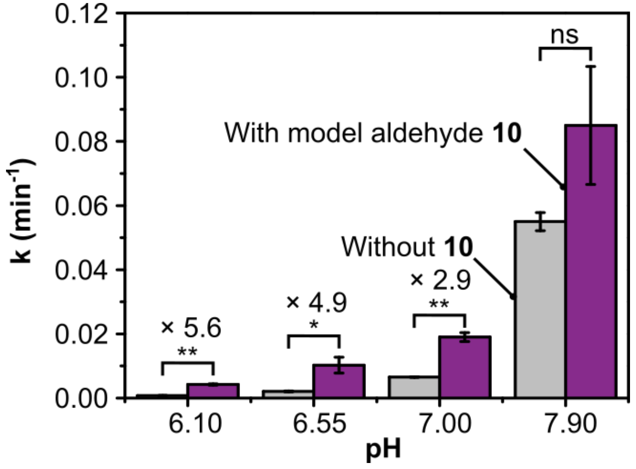
Kinetic Modeling with Aldehyde 10



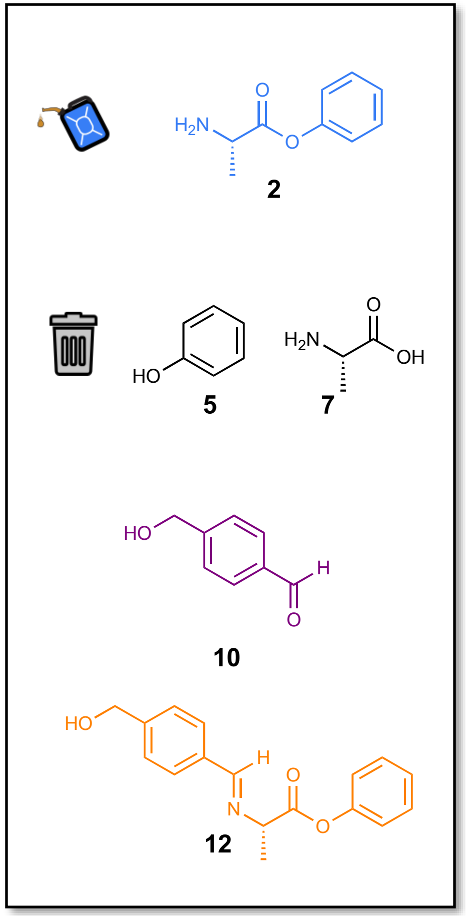
pH 6.10, 6.55, 7.00

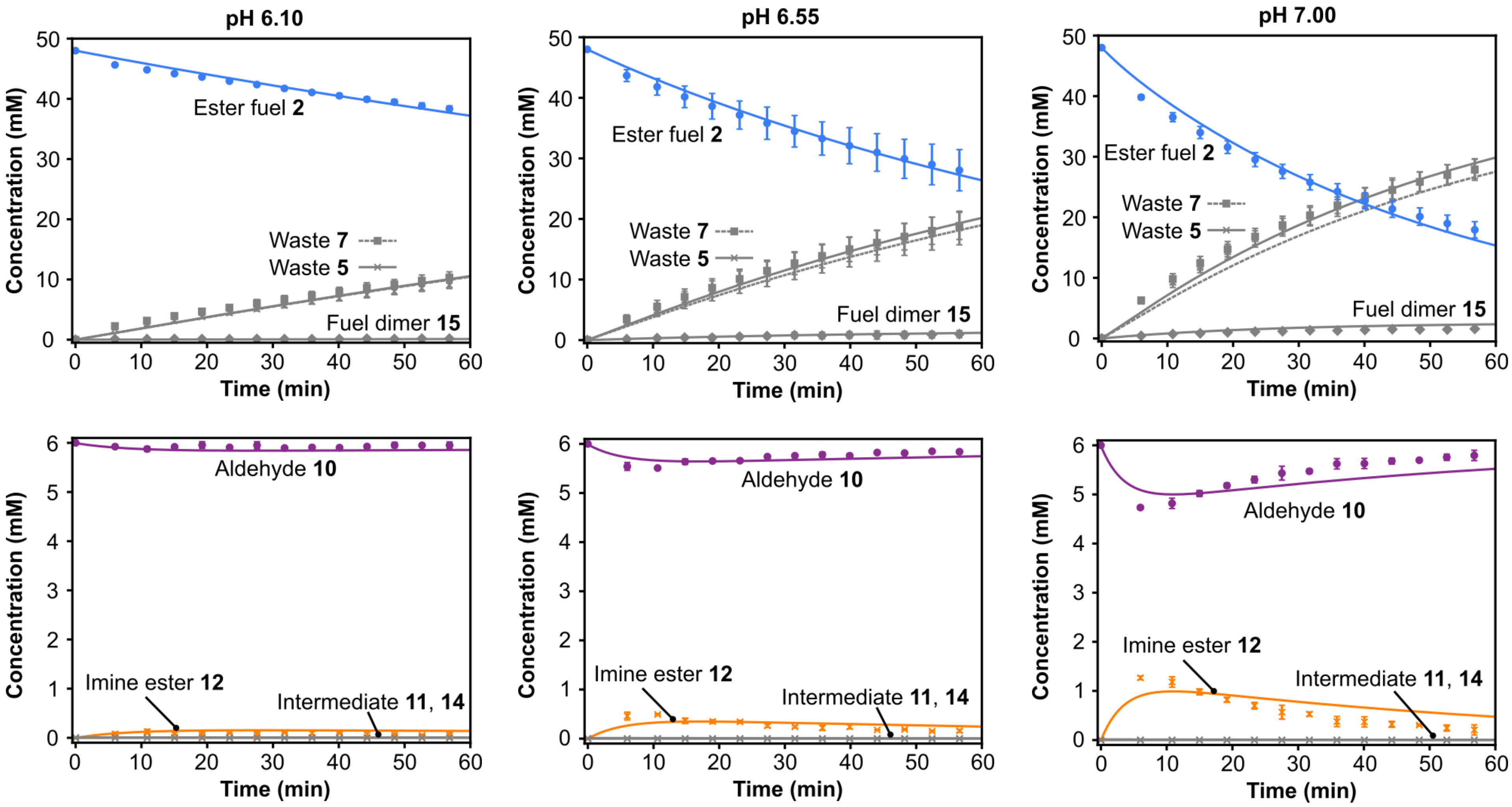


Before adding ester fuel **2** — 3 min — 140 min

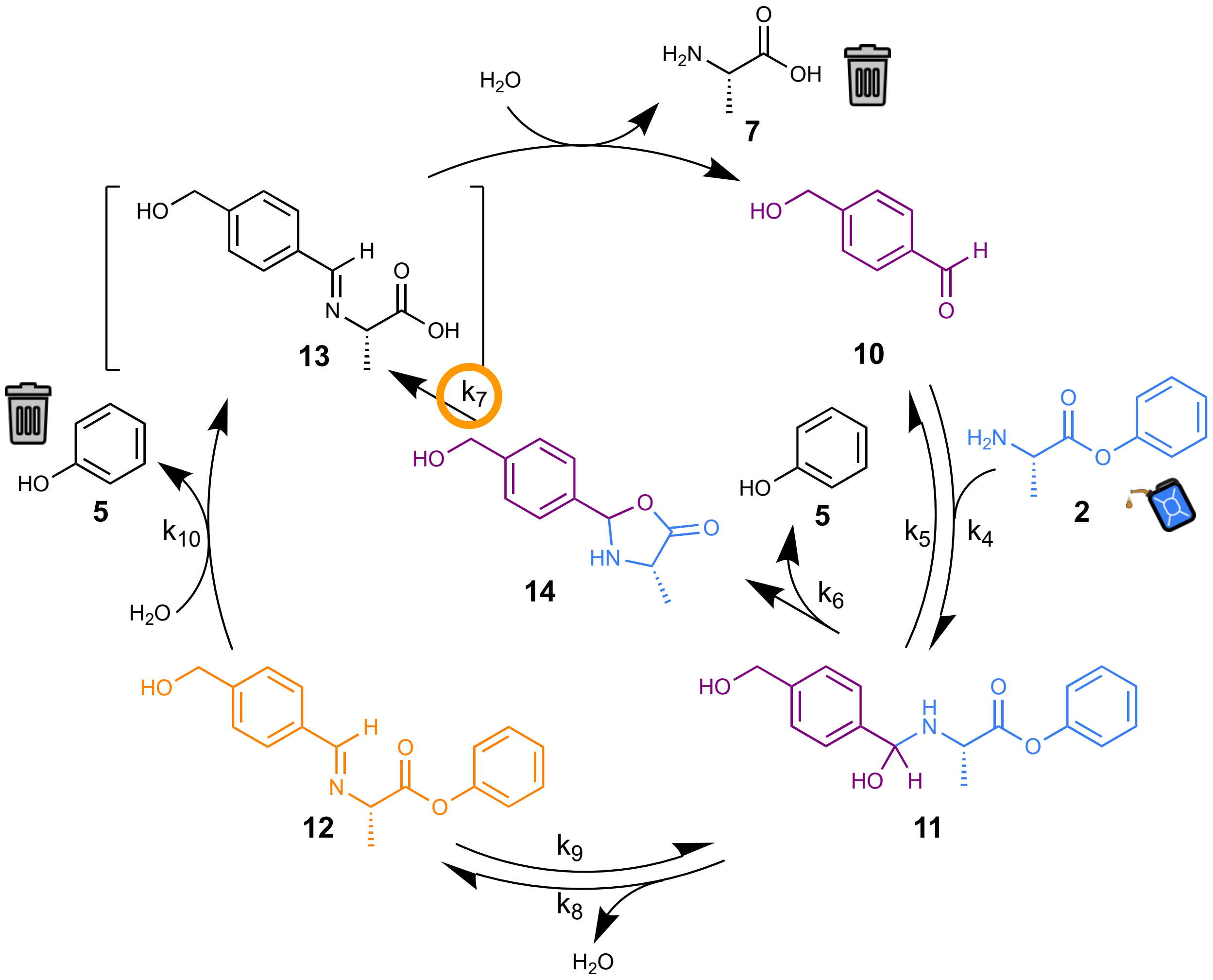


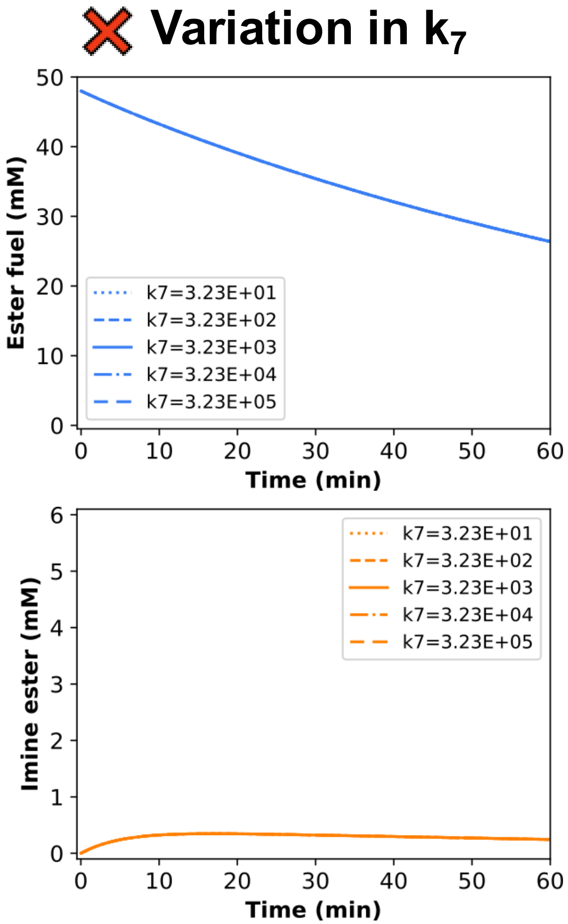
Kinetic Modeling with Aldehyde 10





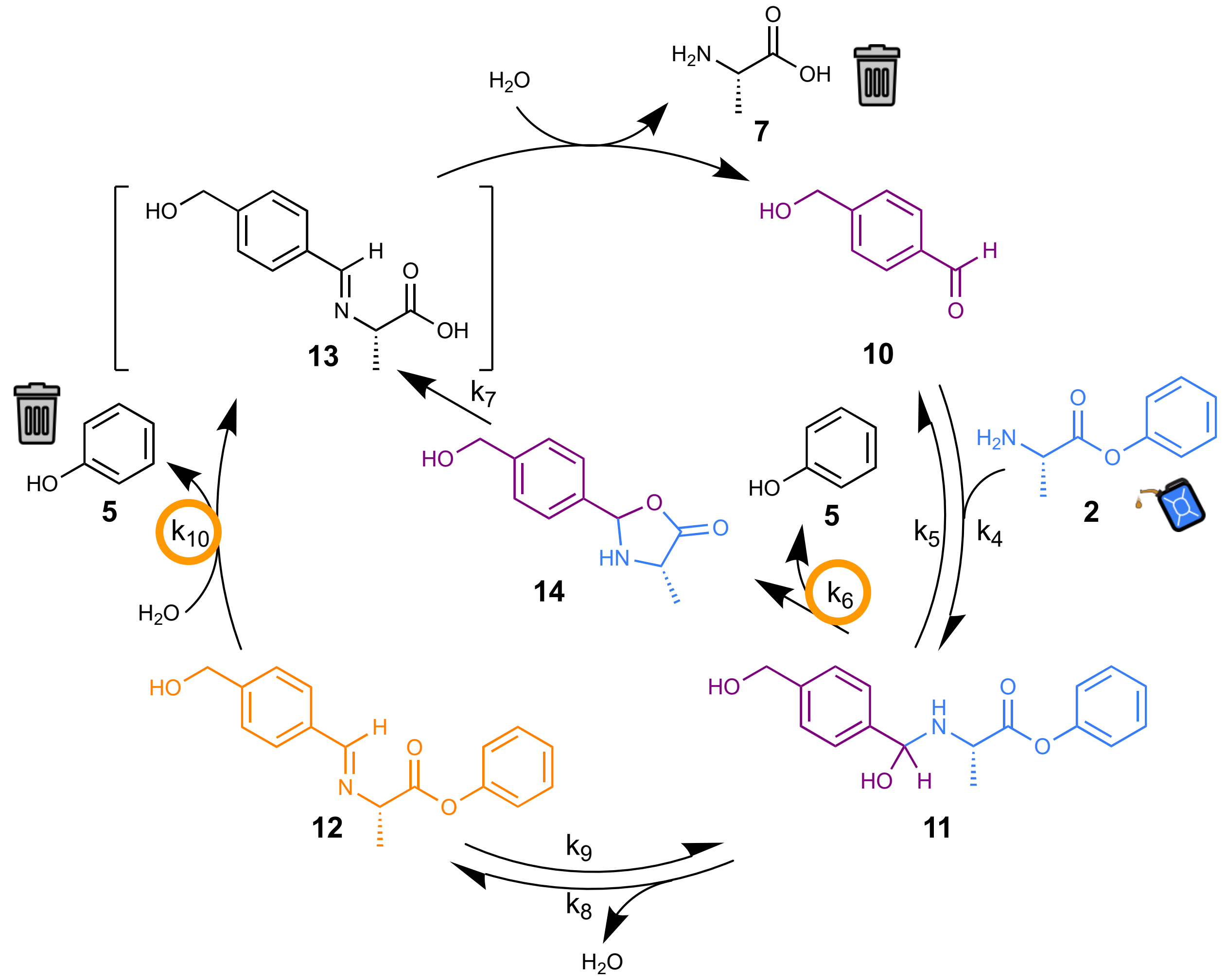
How Does pH Affect Imine Ester Accumulation?

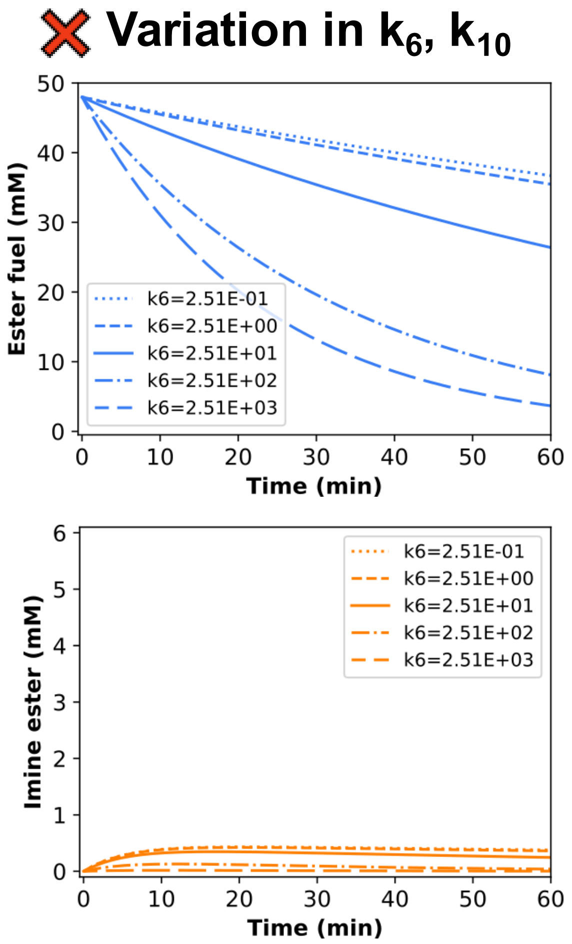




k_7 ↑, no effect on [Imine ester 12]

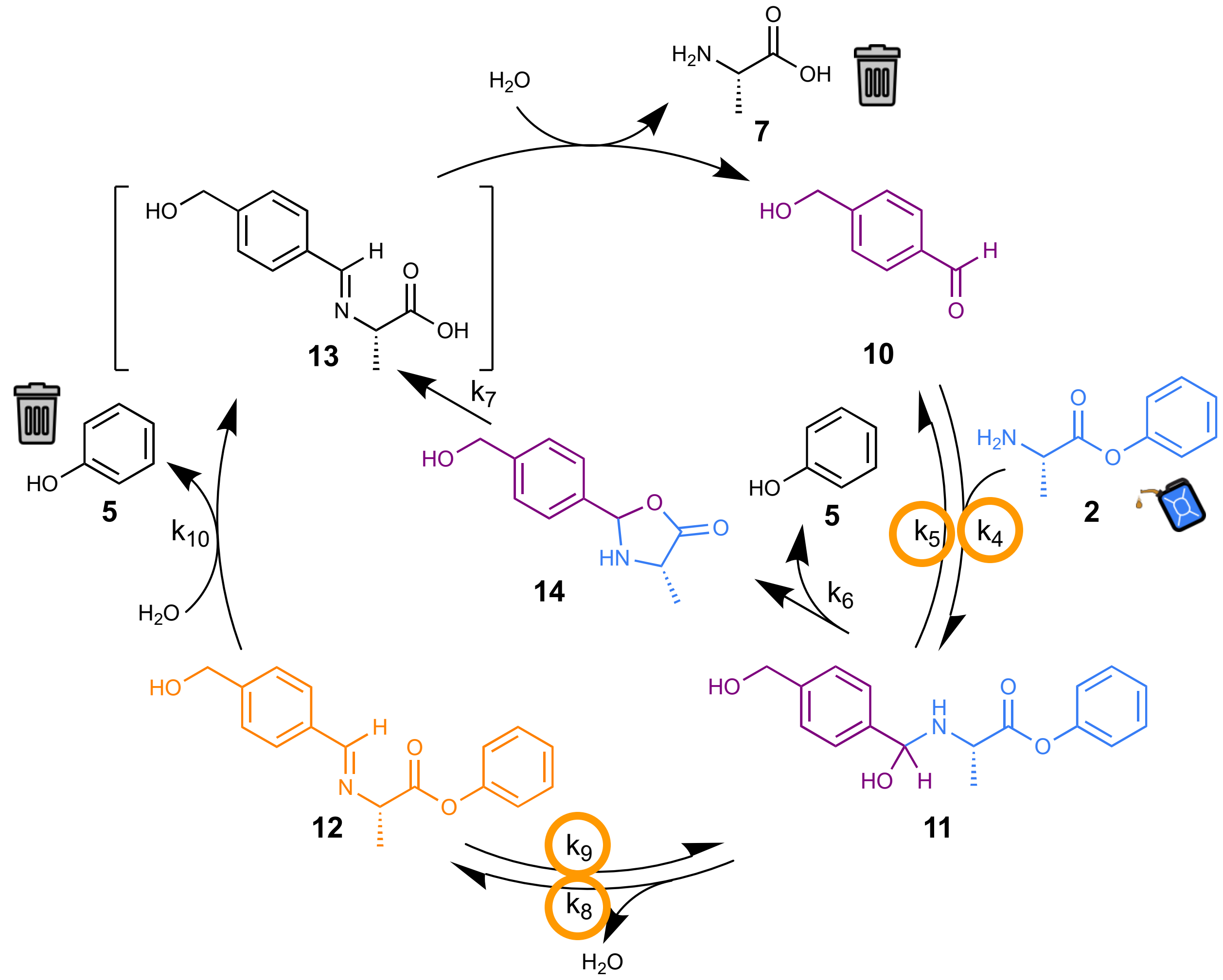
How Does pH Affect Imine Ester Accumulation?



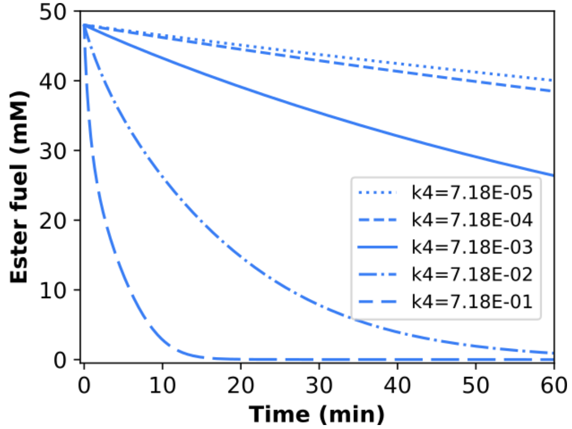


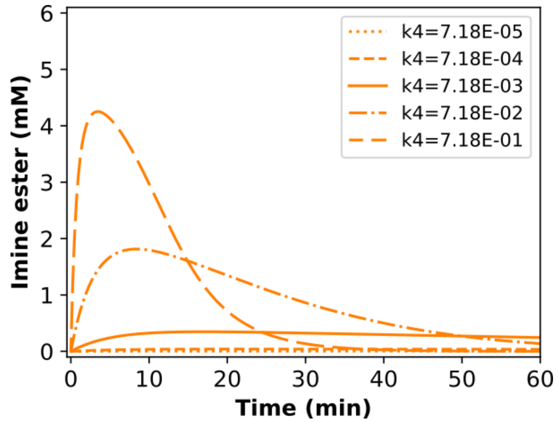
k_6 or k_{10} ↑, [Imine ester 12] ↓

How Does pH Affect Imine Ester Accumulation?



Variation in k_4, k_5, k_8, k_9





Imine formation equilibria shifts to products, [Imine ester 12] ↑