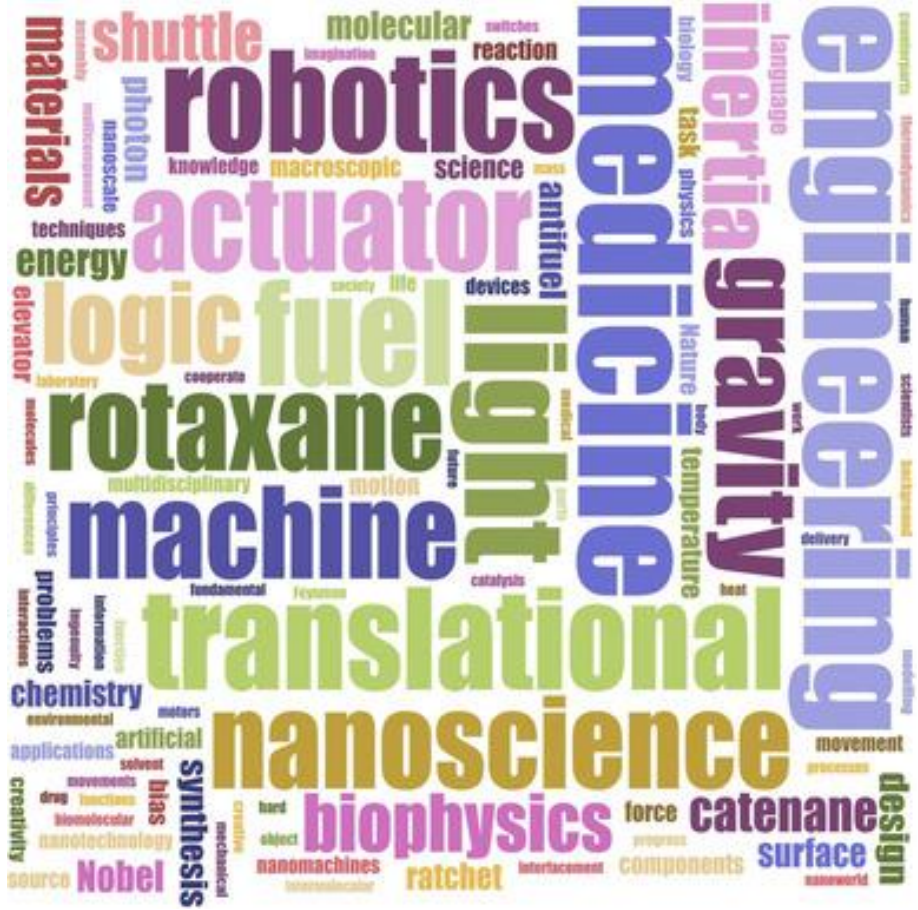
Making and Operating Molecular Machines:

A Multidisciplinary Challenge



"*Nature, in order to carry out the marvelous operations in animals and plants, has been pleased to construct their organized bodies with a very large number of* ***machines****, which are of necessity made up of extremely minute parts so shaped and situated such as to form a marvelous organ, the composition of which are usually invisible to the naked eye, without the aid of microscope*"- Marcello Malpighi (seventeenth century);

As quoted by Marco Piccolino, Nature Rev. Mol. Cell Biology 1, 149-152 (2000).

Marcello Malpighi

(March 10, 1628 - September 30, 1694)

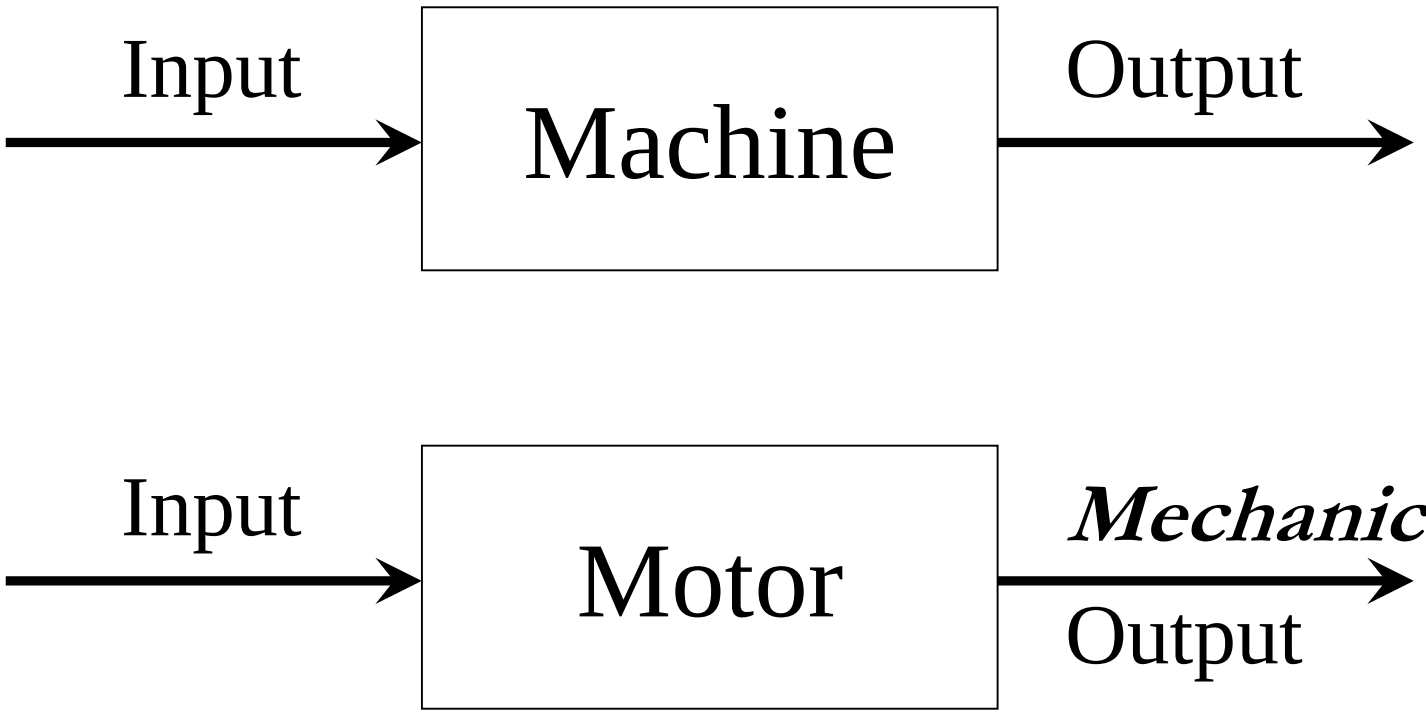
Founder of microscopic anatomy

"*The entire cell can be viewed as a **factory** that contains an elaborate network of interlocking assembly lines, each of which is composed of a set of large protein **machines**.... Why do we call the large protein assemblies that underline cell function protein machines? Precisely because, like machines invented by humans to deal efficiently with the macroscopic world, these protein assemblies contain highly coordinated moving parts*" - Bruce Alberts,

Cell 92, 291 (1998).

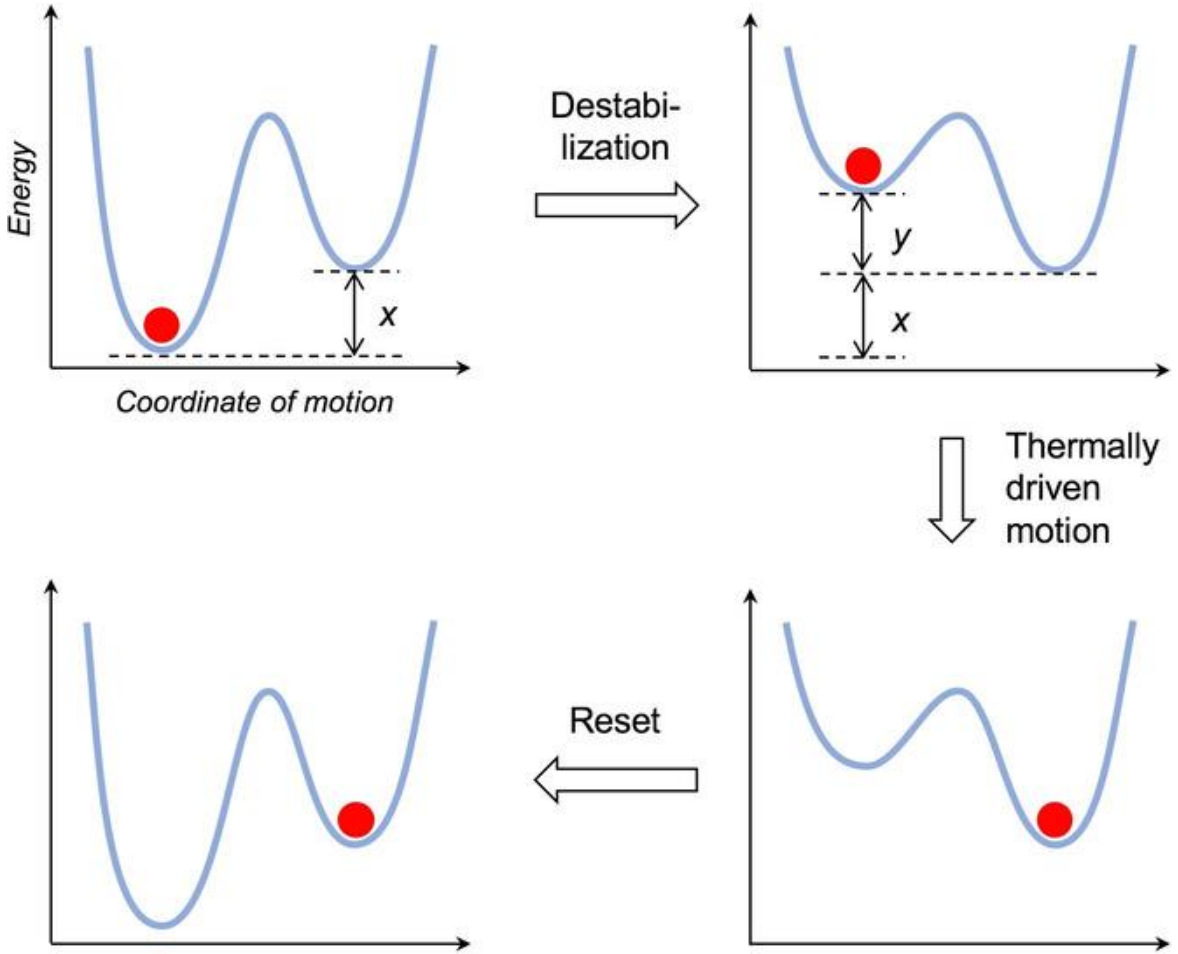
President of the National Academy of Sciences USA (1993-2005)

Editor-in-chief, SCIENCE (March, 2008 -)


Input
Machine
Output
Input
Motor
Mechanical
Output

Molecular versus Macroscopic Machines

While macroscopic machines are made with hard materials and their operation can rely on temperature differences between different parts, molecular machines are made of floppy components and must operate at a constant temperature (dictated by their environment), because heat flows very rapidly over nanometer distances.



Schematic representation of the thermally driven motion of a chemical system biased by an energy input (*y*). The state of the system with respect to the motion coordinate is identified by the position of the red circle on the potential energy curve.

The Energy Issue

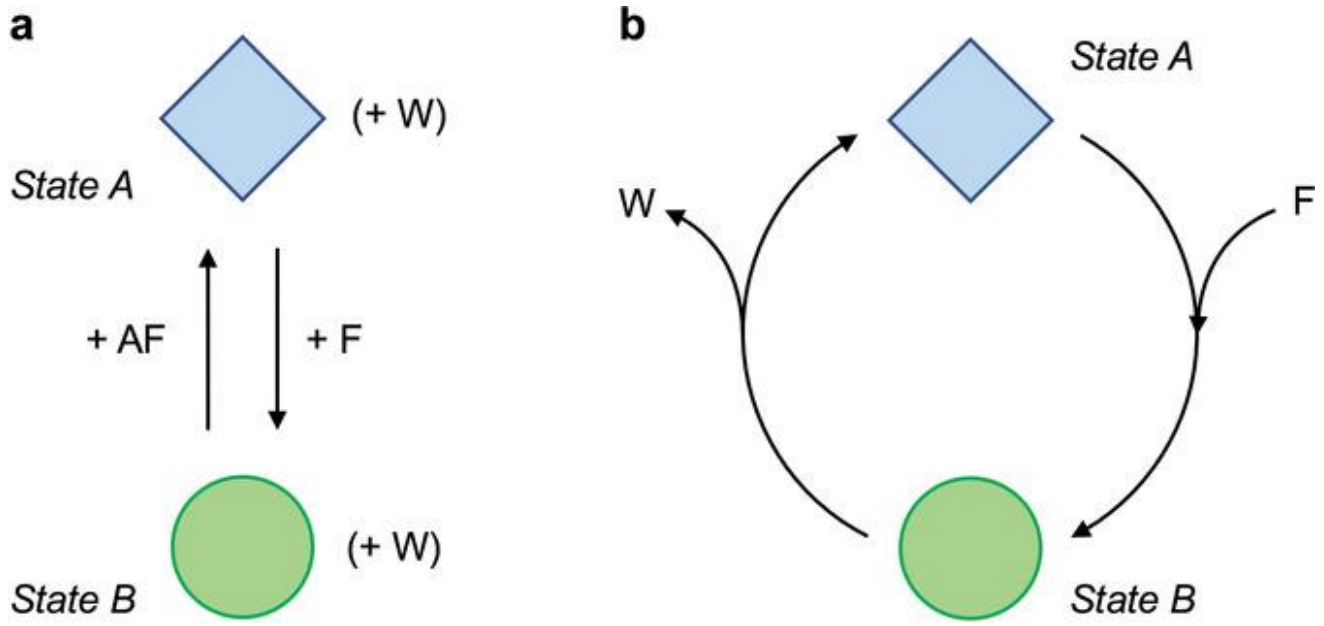
Brownian motion at thermal equilibrium cannot be exploited to achieve directed and controlled movement of a molecular machine.

Thus, molecular machines need an external input of energy to operate.

ChemistryOpen 2018, 7, 169 –179

Chemical Energy

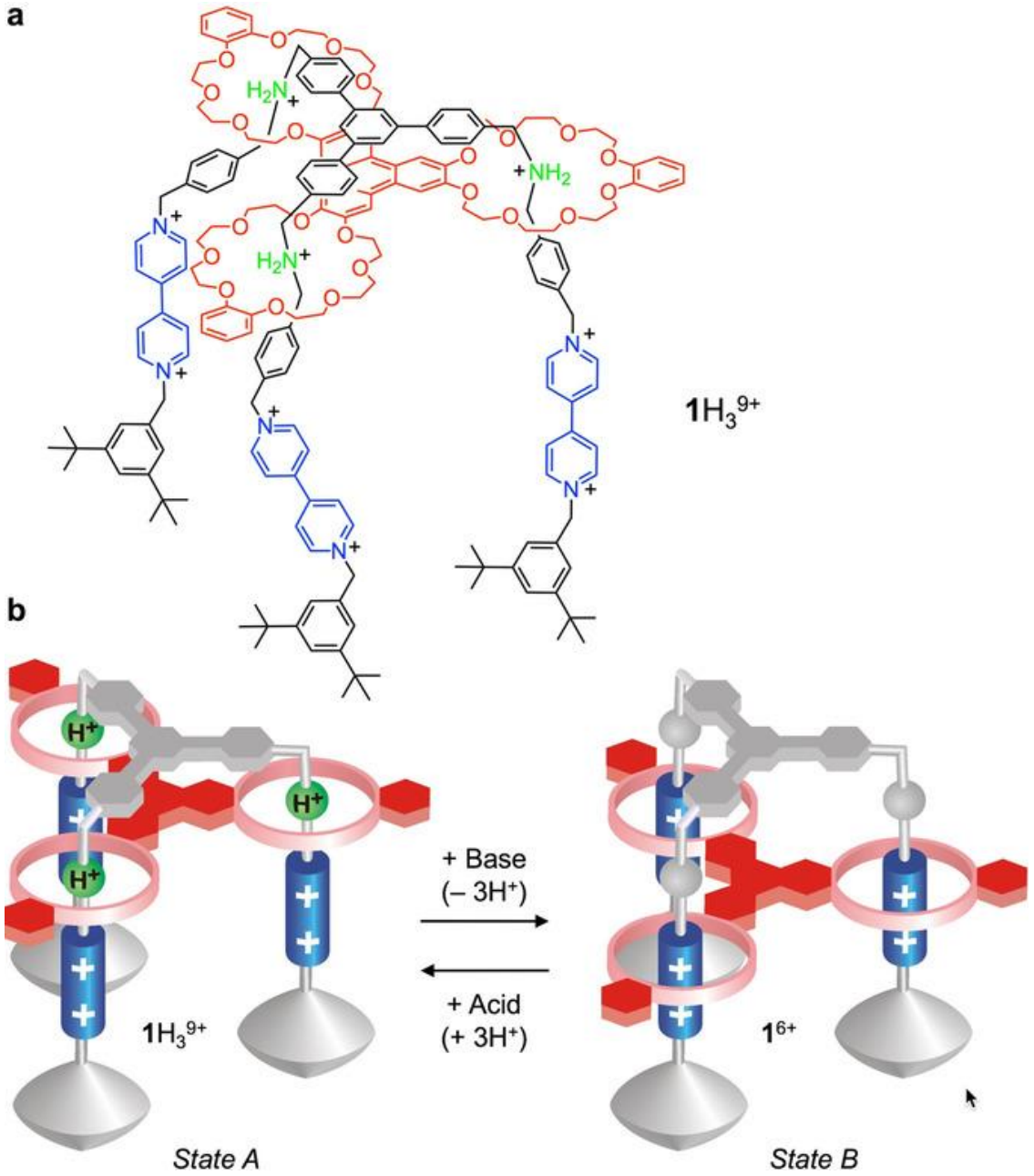
Chemically powered molecular machines require that the fuel be delivered where and when necessary. The reaction of the fuel will generate products (“waste”) which, even if they do not interfere with the operation of the machine, will accumulate in the reaction medium, unless they are appropriately removed.



A mechanical molecular switch that uses a fuel (F) and an anti-fuel (AF) to convert between two structurally different equilibrium states. Repeated switching (cycling) between the states requires the sequential addition of reactants. b) An autonomous chemically driven molecular machine that exploits the catalytic decomposition of a fuel to perform the transformation between two structurally different states. The process can continue indefinitely without the intervention of an operator as long as the fuel is available. Waste products (W) are formed in both cases.

ChemistryOpen 2018, 7, 169 –179

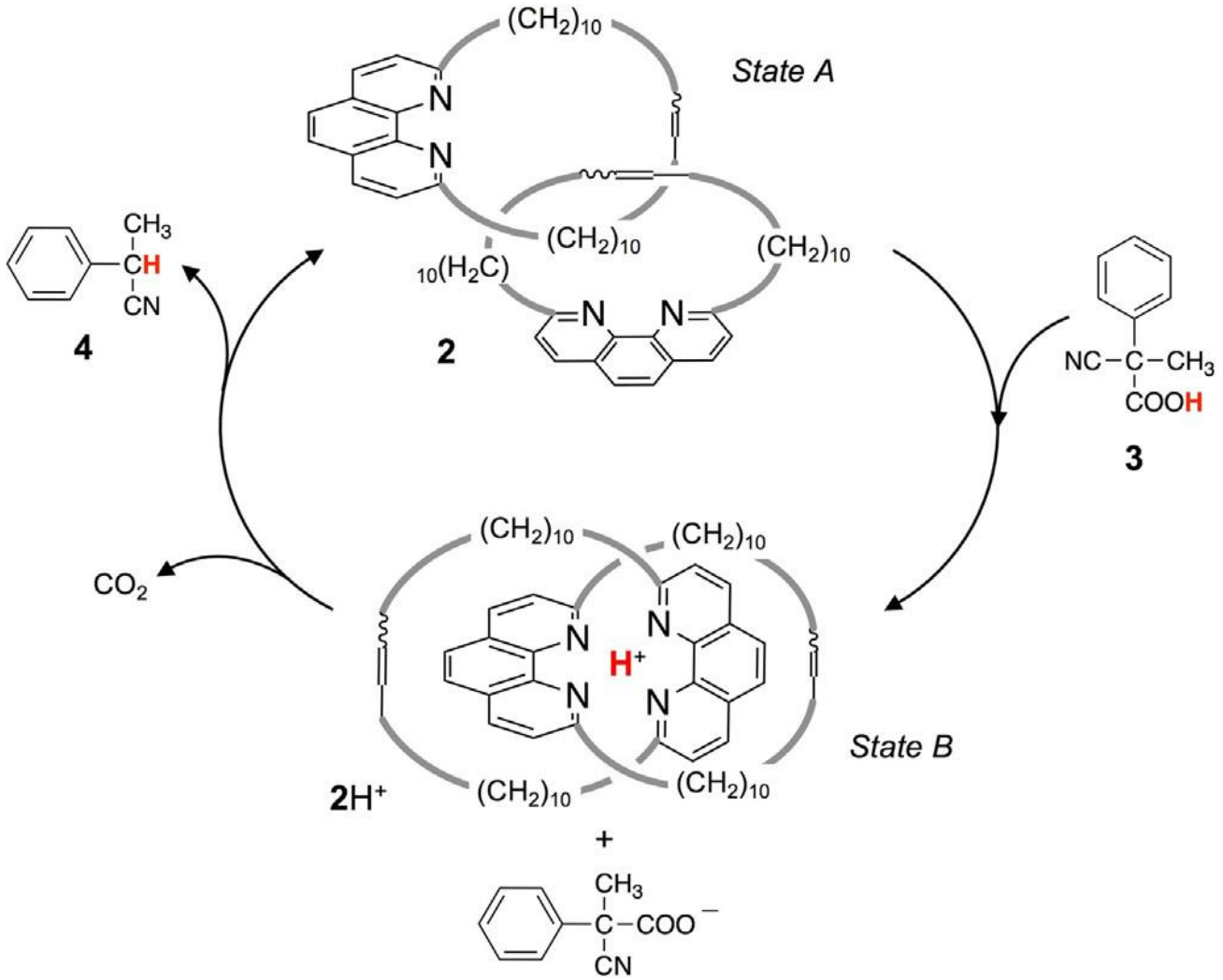
Structure formula (a) and base/acid-triggered operation scheme (b) of molecular elevator 1 H39+



When an appropriate base is added, the ammonium sites of **1** H_3^{9+} are deprotonated and, as a consequence, the platform moves to the "lower" level, with the three rings surrounding the bipyridinium sites (Figure 3 b, state B). Such a structure of $\mathbf{1}^{6+}$ is stabilized by charge-transfer interactions between the π-electron-rich aromatic units of the platform and the π-electron-poor- bipyridinium units of the framework. Upon successive addition of acid, the ammonium sites are restored and the platform returns to the "upper" level. The elevator motion, which can be followed by NMR spectroscopy, electrochemistry, and absorption and fluorescence spectroscopy, is quantitative and can be repeated by the sequential addition of a basic fuel and an acid anti-fuel

J. D. Badjic, V. Balzani, A. Credi, S. Silvi, J. F. Stoddart,
Science 2004,
303, 1845–1849;

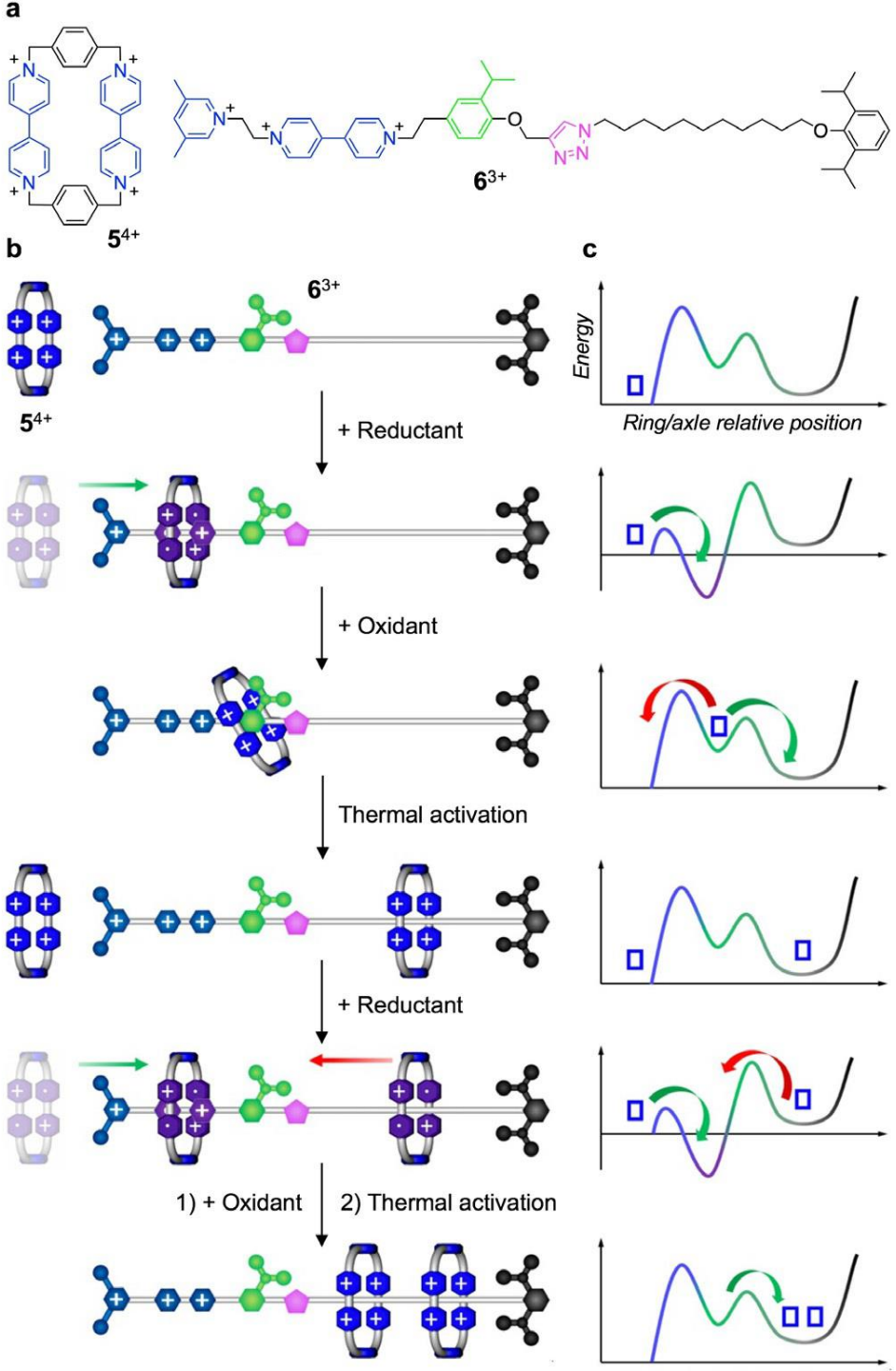
A step forward towards the development of fully artificial molecular machines that can exploit autonomously a chemical fuel is the system shown here



Schematic representation of the switching cycle of catenane 2, activated by the decomposition of the fuel 3 which is converted into waste products carbon dioxide and 4

In solution, [2]catenane 2 assumes a co conformation in which the two bulky and rigid phenanthroline moieties are located away from each other, state A

The addition of 2-cyano-2-phenylpropanoic acid (3) causes the protonation of the catenane (2H+), which is accompanied by a rearrangement of the molecular rings, state B



This is the case for the chemically driven supramolecular pump
illustrated in Figure 5,[31] whose components are the tetra cationic macrocycle 5^{4+} and the tricationic axle 6^{3+}.

In water, the chemical reduction of the bipyridinium units of the ring and the axle promotes their threading by passage of the ring over the positively charged 3,5-dimethylpyridinium end group.

Successive oxidation causes the buildup of an electrostatic repulsion between the 5^{4+} ring and the bipyridinium site on 6^{3+}.

The ring, however, cannot dethread because of the now increased electrostatic barrier represented by the 3,5-dimethylpyridinium group; it can only move towards the ring collecting alkyl chain.

In the successive reduction step, the presence of the isopropylphenylene group makes the return of the threaded macrocycle slower than the entrance of a second ring which, upon oxidation, is pushed on the ring collecting chain.

Such a reduction-oxidation cycle can be repeated indefinitely, although only two rings have been pumped so far experimentally.

C. Cheng, P. R. McGonigal, S. T. Schneebeli, H. Li, N. A. Vermeulen, C. Ke, J. F. Stoddart, Nat. Nanotechnol. 2015, 10, 547–553.

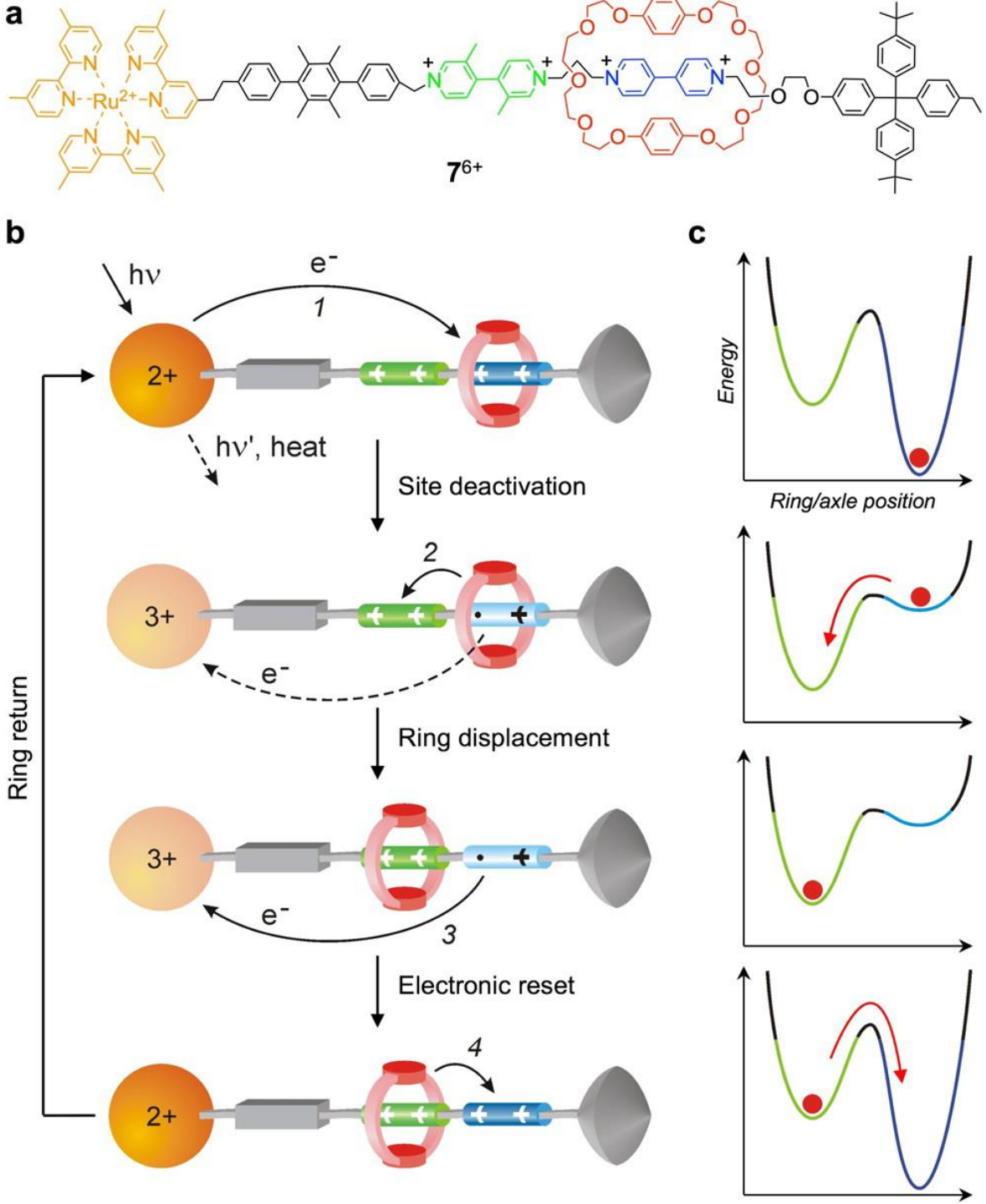
Light Energy

Although solar radiation is the only energy source for our planet, in Nature sunlight—or, in general, light energy—is not directly converted into mechanical movements.

Rather, it is used to produce chemical fuels (e.g., ATP) suitable for powering biomolecular machines.

Light energy, however, can cause reactions that involve large structural changes in molecular systems. A simple example of this kind of photochemical reactions is the light-induced transformation between the E and Z isomers of molecules that contain a -N=N- or -C=C double bond.

In multicomponent species, photoinduced electron- or proton-transfer reactions can also cause a major displacement of the molecular parts.



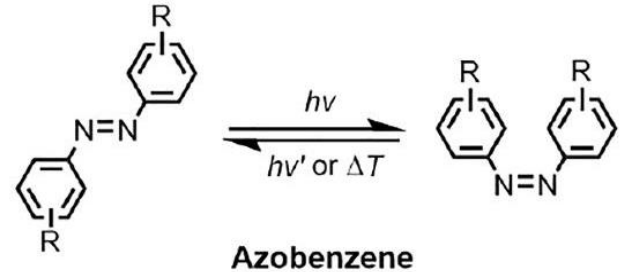
In the stable co-conformation of the rotaxane, the ring component— an electron donor crown ether—encircles the primary electron acceptor site—a 4,4'-bipyridinium-type unit—because this site is a better electron acceptor than the other one—a 3,3'-dimethyl-4,4'-bipyridinium-type unit.

The mechanism for photoinduced ring displacement consists of four steps. In solution at room temperature, excitation of the photoactive RuII-based moiety with visible light causes the transfer of an electron from this unit to the primary electron acceptor site (step 1), which becomes deactivated. As a consequence, the ring moves to encircle the secondary site step 2).
A back electron transfer from the free reduced site to the oxidized Ru-based unit (step 3) restores the electron acceptor ability of the former site. As a consequence of such an electronic reset, the ring returns to the original primary acceptor site (step 4). The switching cycle is thus completed and therotaxane is ready to process another photon.

The automatic thermally driven reset of the molecular machine enables its autonomous operation under the supply of light energy

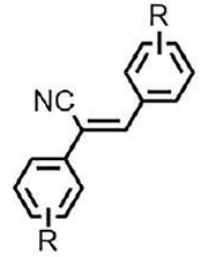
Molecular Switches

cis-trans isomerism

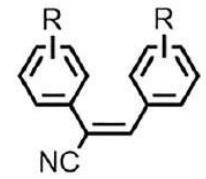


hv ⇌ *hv'* or Δ*T*

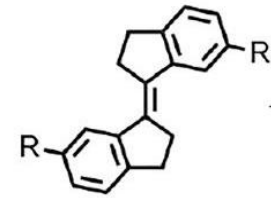
Azobenzene



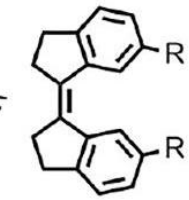
hv ⇌ *hv'* or Δ*T*



Cyanostilbene

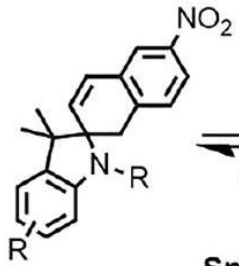


hv ⇌ *hv'*

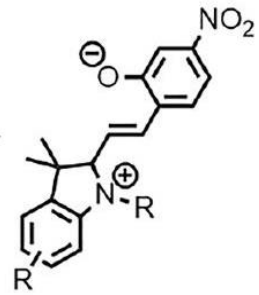


Stilbene

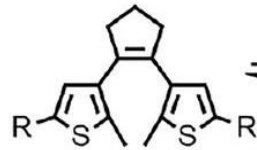
dynamic chemical bonds



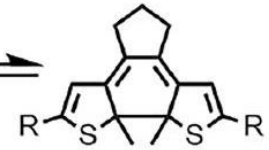
hv ⇌ *hv'* or Δ*T*



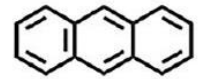
Spiropyran



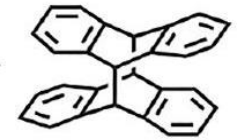
hv ⇌ *hv'*



Dithienylethene

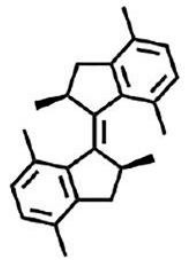


hv ⇌ *hv'* or Δ*T*

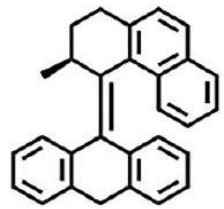


Anthracene

Feringa's Alkene Molecular Motors

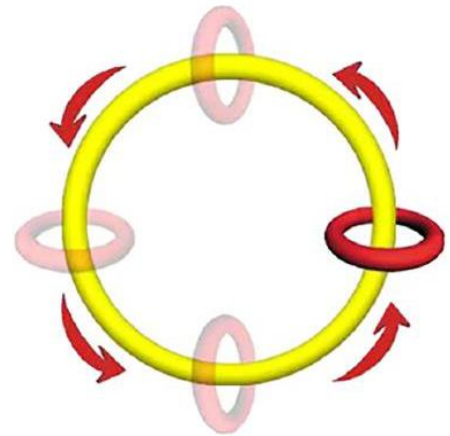


First-Generation

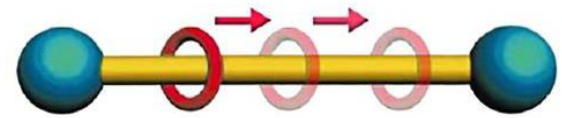


Second-Generation

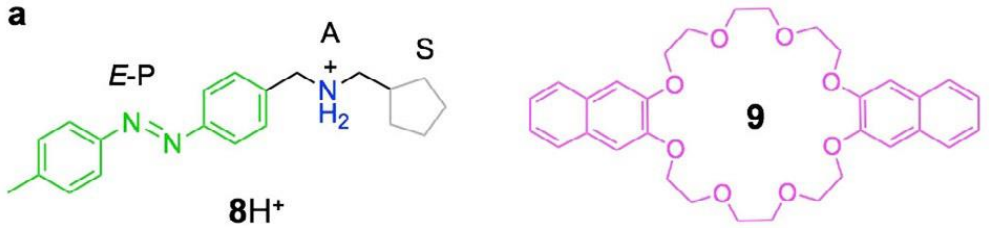
Catenane Molecular Motor

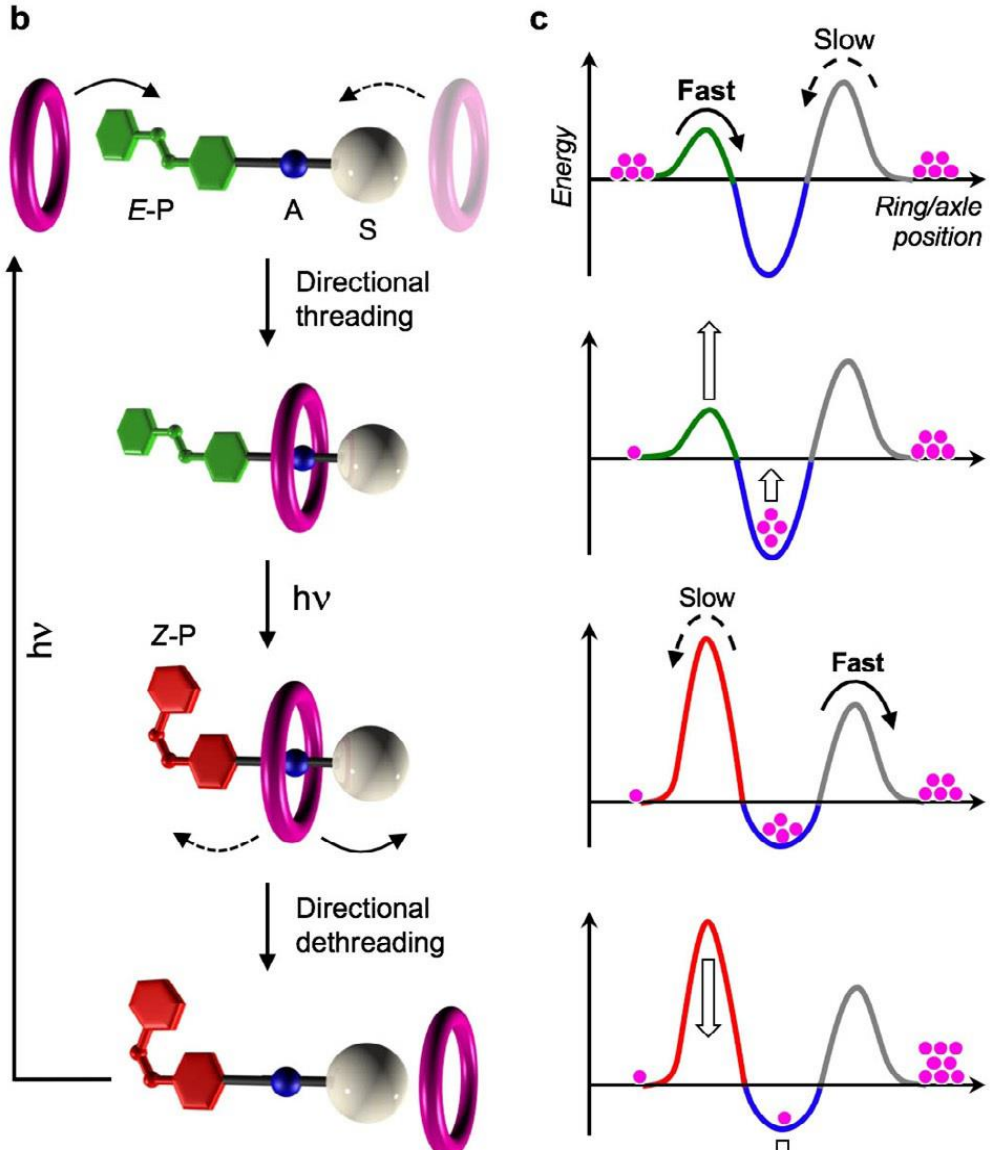


Rotaxane Molecular Shuttle



artificial molecular pump





The photoisomerization of azobenzene between E and Z form, combined with the self-assembly of a pseudorotaxane species, was recently exploited to obtain an autonomous supramolecular pump powered by light energy.

The components are the non-symmetric molecular axle 8H+, comprising an azobenzene photoswitchable extremity (P), an ammonium recognition site (A) and a photoinactive cyclopentyl end (S), and the crown ether ring 9.

In a dichloromethane solution at room temperature, there is a driving force for the assembly of a pseudorotaxane in which 9 encircles the ammonium site of E-8H+ on account of hydrogen bonding interactions.

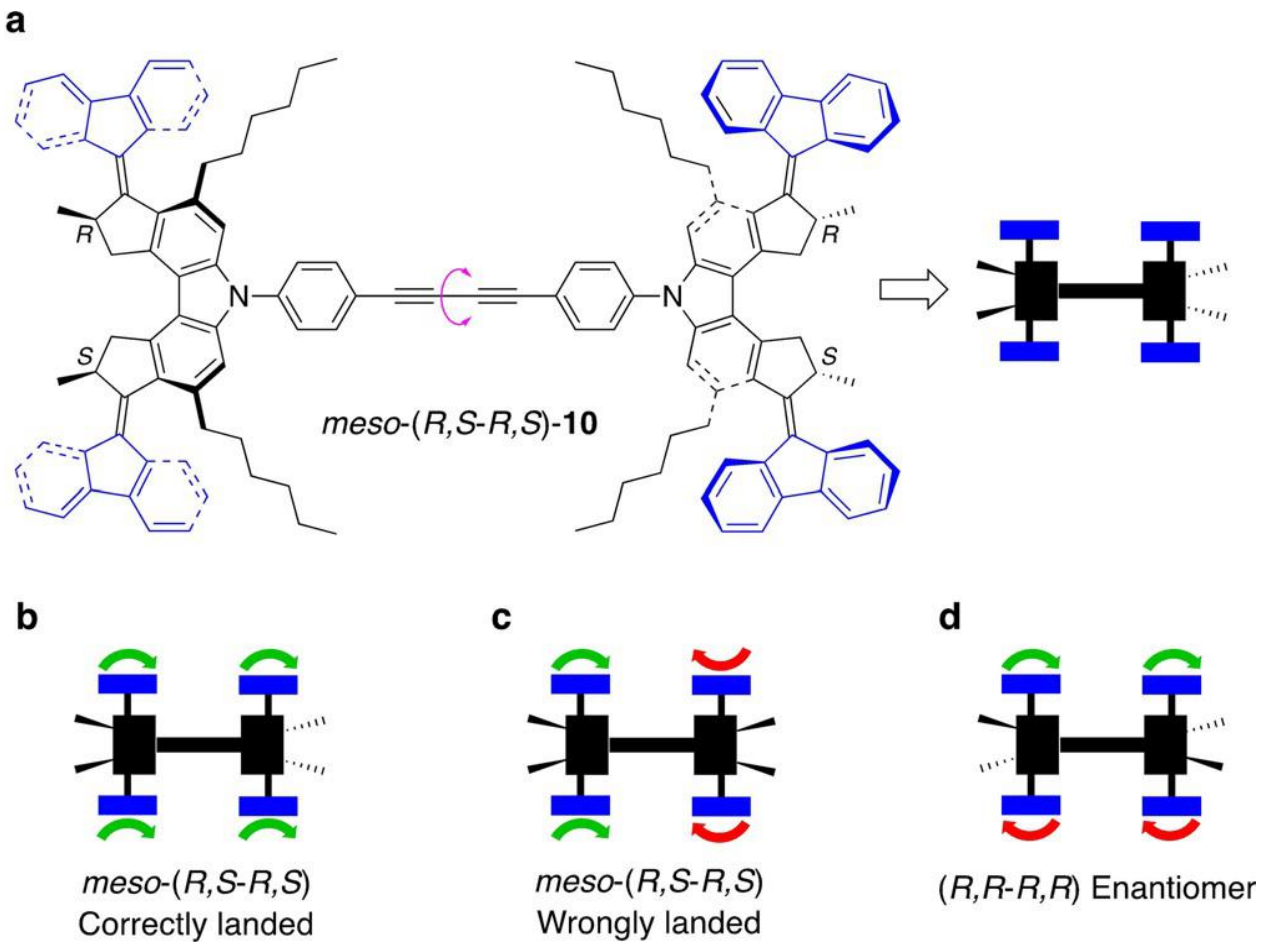
As the P-end in its E configuration exhibits a much smaller hindrance than the S-end for the transit of the ring, there is a strong kinetic preference for threading through the P-end, thus dictating the direction of the first step.

G. Ragazzon, M. Baroncini, S. Silvi, M. Venturi, A. Credi, Nat. Nanotechnol. 2015, 10, 70–75.

Electrical Energy

Electrical energy can also be conferred to chemical systems without causing redox processes. Indeed, individual molecules lying on a surface can be electronically and/or vibrationally excited by the application of an appropriate voltage with a local probe, such as the tip of a scanning tunneling microscope (STM). In recent years, this approach was followed to accomplish directed motion of single molecules on surfaces.

“nanocar”



rotational freedom around the bis-alkyne C-C

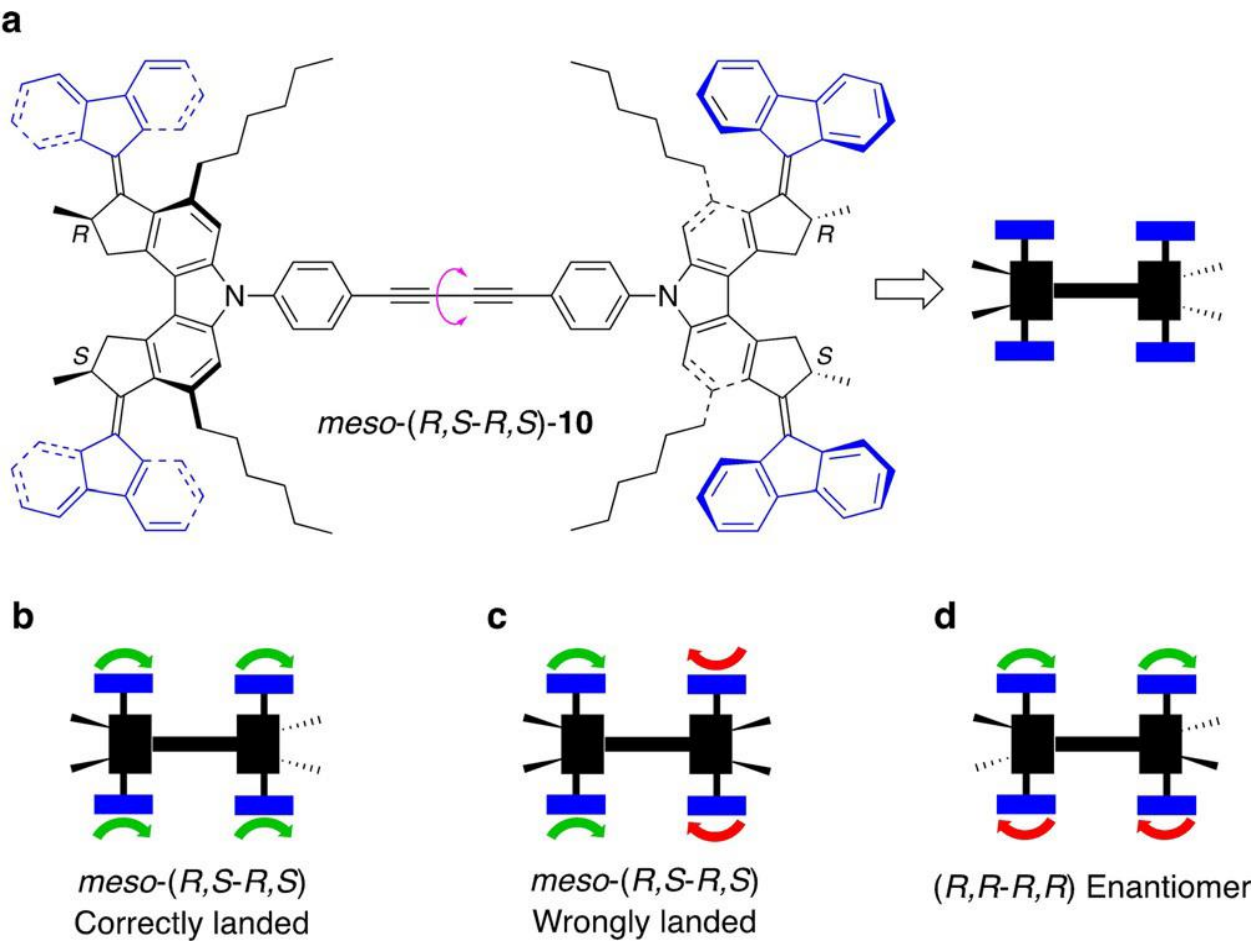
An impressive example of this kind is the “nanocar” molecule 10 (Figure 8a), that consists of four overcrowded alkene rotary motor units as the wheels, mounted on a rigid bis(phenylene ethynylene) chassis.

In each wheel, electronic (e.g., light) and vibronic (e.g., thermal) excitation can induce, respect-tively, configurational and conformational changes that ultimately cause the unidirectional rotation of the fluorene moiety around the C=C bond.

It was thus hypothesized that the STM tip, by inducing electronic and vibronic excitations, could trigger the isomerization processes necessary for the rotary motion of the wheels of 10, and concurrently provide single molecule imaging. To achieve directed motion on the surface, the four wheels need to move in a conrotatory manner ; as the direction of rotation of each motor unit is dictated by its chirality, the meso-(R,S-R,S) isomer of 10 is the appropriate nanocar candidate.

Another necessary condition for translation is that the energy dissipated in the isomerization steps be greater than that associated with the adsorption of the molecule on the surface. In fact, the adsorption energy of 10 was expected to be modest because the strained helical structure of the wheels prevents the aromatic moieties from aligning parallel to the surface.

“nanocar”



rotational freedom around the bis-alkyne C-C

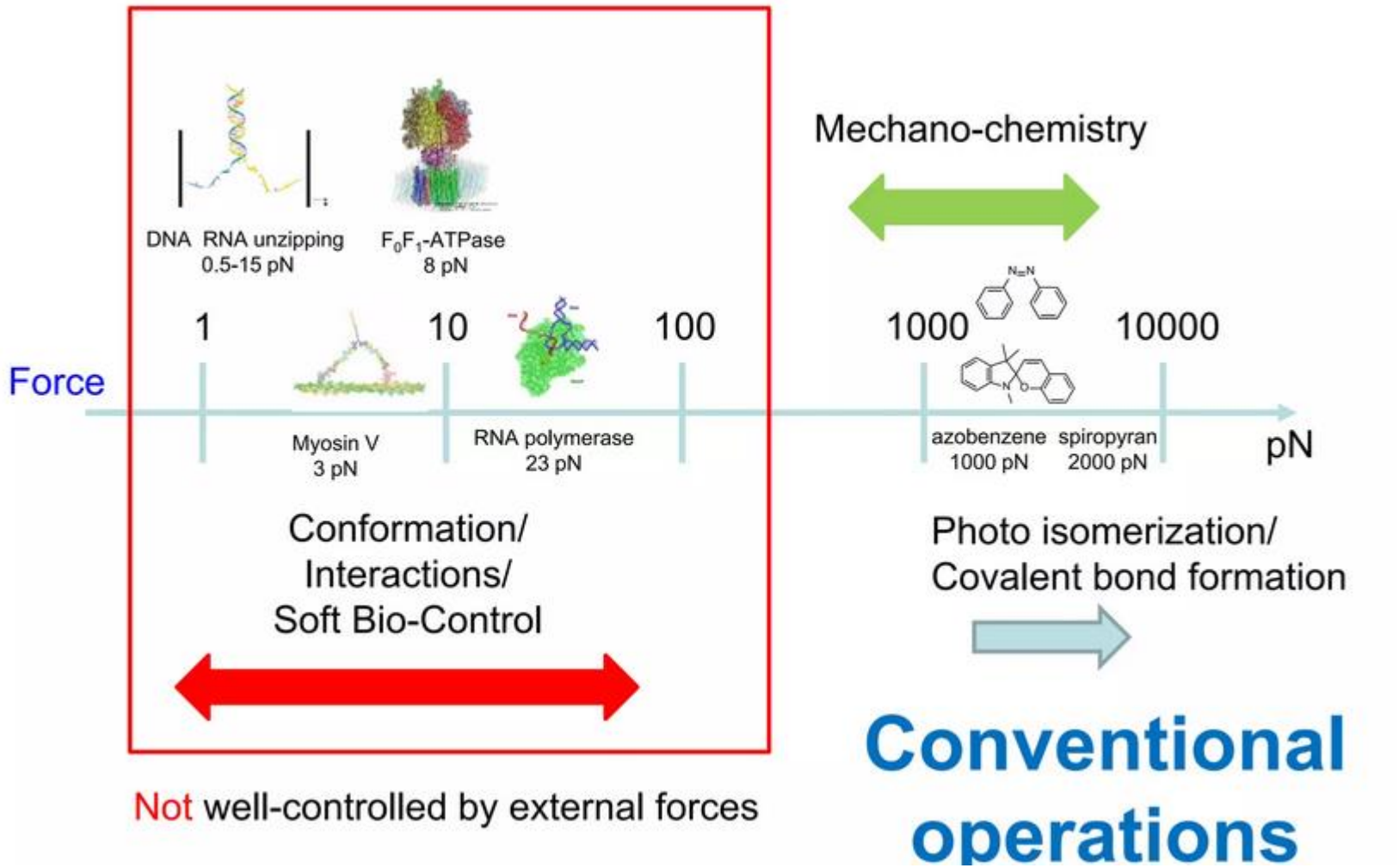
Upon ten excitation steps, a translation of 6 nm across the surface was observed for some of the imaged molecules. Not all nanocars, however, were found to move directionally.

Because of the rotational freedom around the bis-alkyne C@C bond of the chassis (pink arrow in Figure 8a), which is locked upon deposition, some molecules became adsorbed in a geometry such that the rotations of the wheels cancel out, thereby preventing translation.

The electrically driven movement of 10 was further supported by the investigation of the individual (R,R-R,R) or (S,S-S,S) enantiomers, deposited on the surface from the racemic mixture, which were found to spin and wander on the surface.

Such a behavior can be explained considering that the disrotatory motion of the motor units on opposite sides of the nanocar ideally causes the molecule to spin (Figure 8d); in a nonideal case, the spinning motion occurs along with random translational motion.

How to control a molecule: Unexplored region

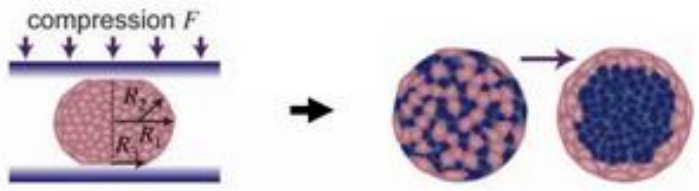


Forces are measured in Newtons (N); one Newton corresponds typically to the weight of a cup of tea. Of course, forces at the molecular scale are far smaller. They turn out to be on the order of picoNewtons (pN). If you divide a Newton by one million you get a microNewton; if you divide a microNewton by a million, you get a picoNewton ($1pN = 10^{-12}$ N).

Type of Force	Example	Rupture Force
Breaking of a covalent bond	C–C	≡1600 pN
Breaking of a noncovalent bond.	Biotin/streptavidin	≡160 pN
Breaking of a weak bond.	Hydrogen bond	≡4 pN
Langevin force	on E-coli	0.01 pN (1s)
Stretching dsDNA	to 50% relative extension	0.1 pN
Developped by a molecular motor	Kinesin walking on microtubule	5 pN (max)

Small Forces (pN) for Bio-functions

Cell migration (infiltration of cancer)

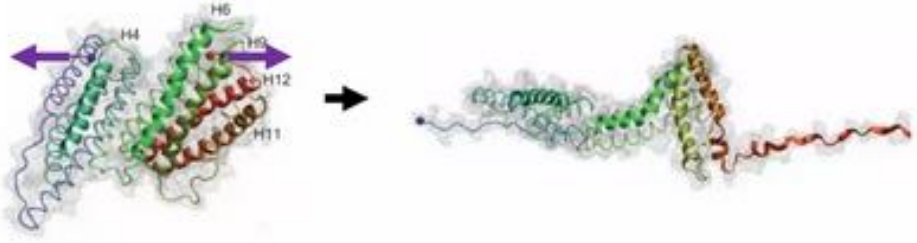


D. G. Rodriguez *et al.*
Science, **2012**, *338*, 910.

Cell differentiation (ES/iPS)

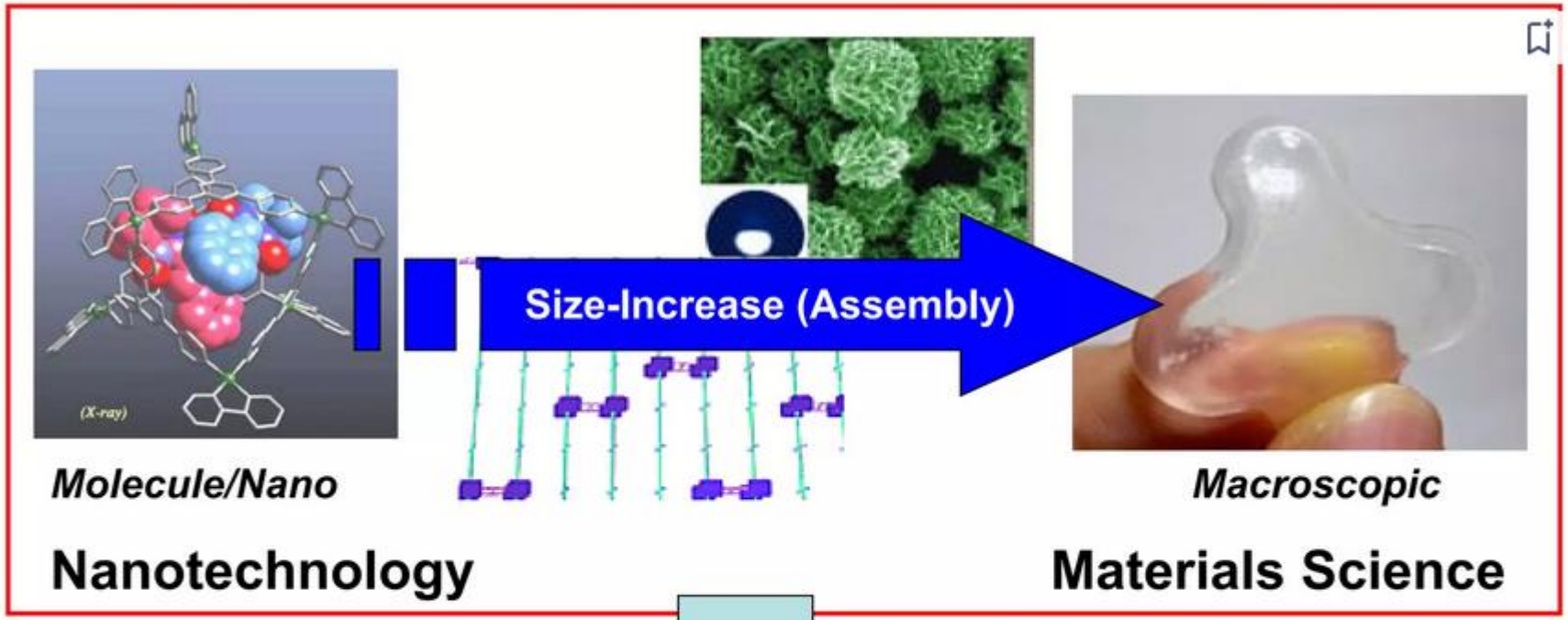
D. E. Discher *et al.*
Science, **2005**, *318*, 1139.

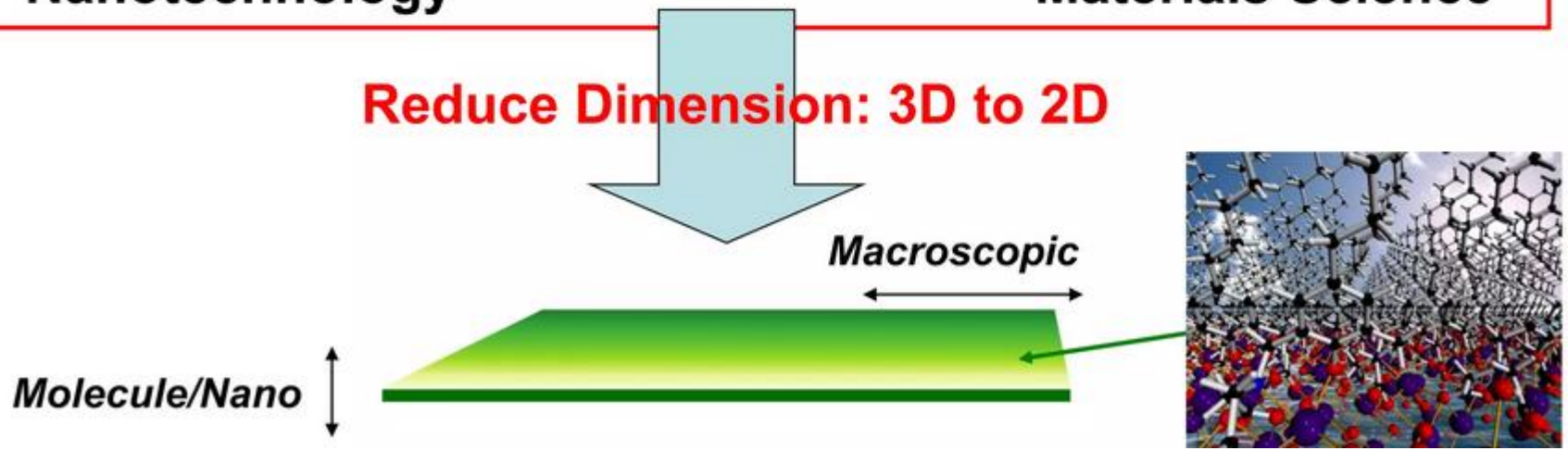
Protein conformational change (change in ligand binding and enzymatic activity)



M. P. Sheetz *et al.*
Science, **2009**, *323*, 638.

…. artificial controls under challenges

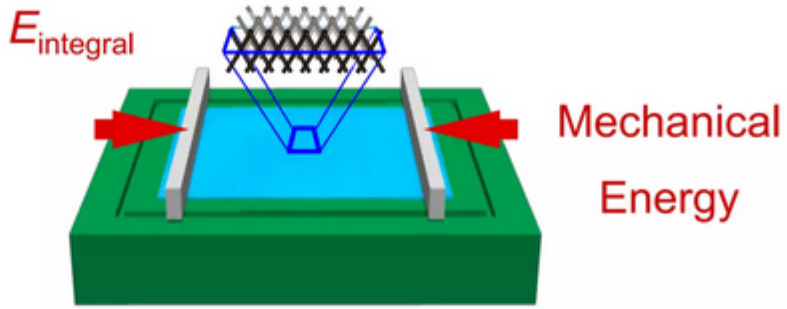


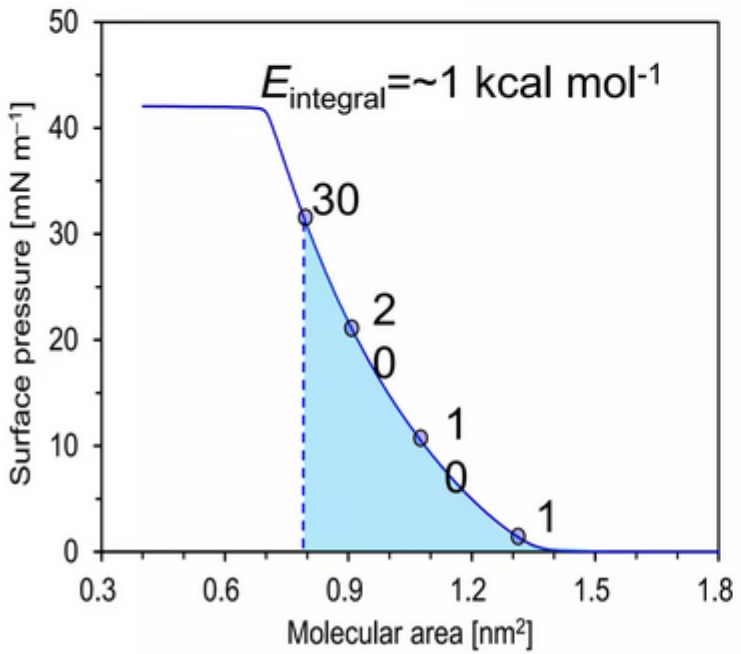


Nanotechnology & Materials Science

Force/Energy at the Air-Water Interface: Langmuir Monolayer System

megapascal (*MPa*) : 1 *MPa* = 10^6 Pa = 1 000 000 Pa = 1 000 000 N m-2 = 1 N mm^{-2}





Surface pressure (*F*): 1-70 mN/m

Estimated force per molecule

≅ 0.5-35 pN

Estimated pressure

≅ 0.5-35 MPa

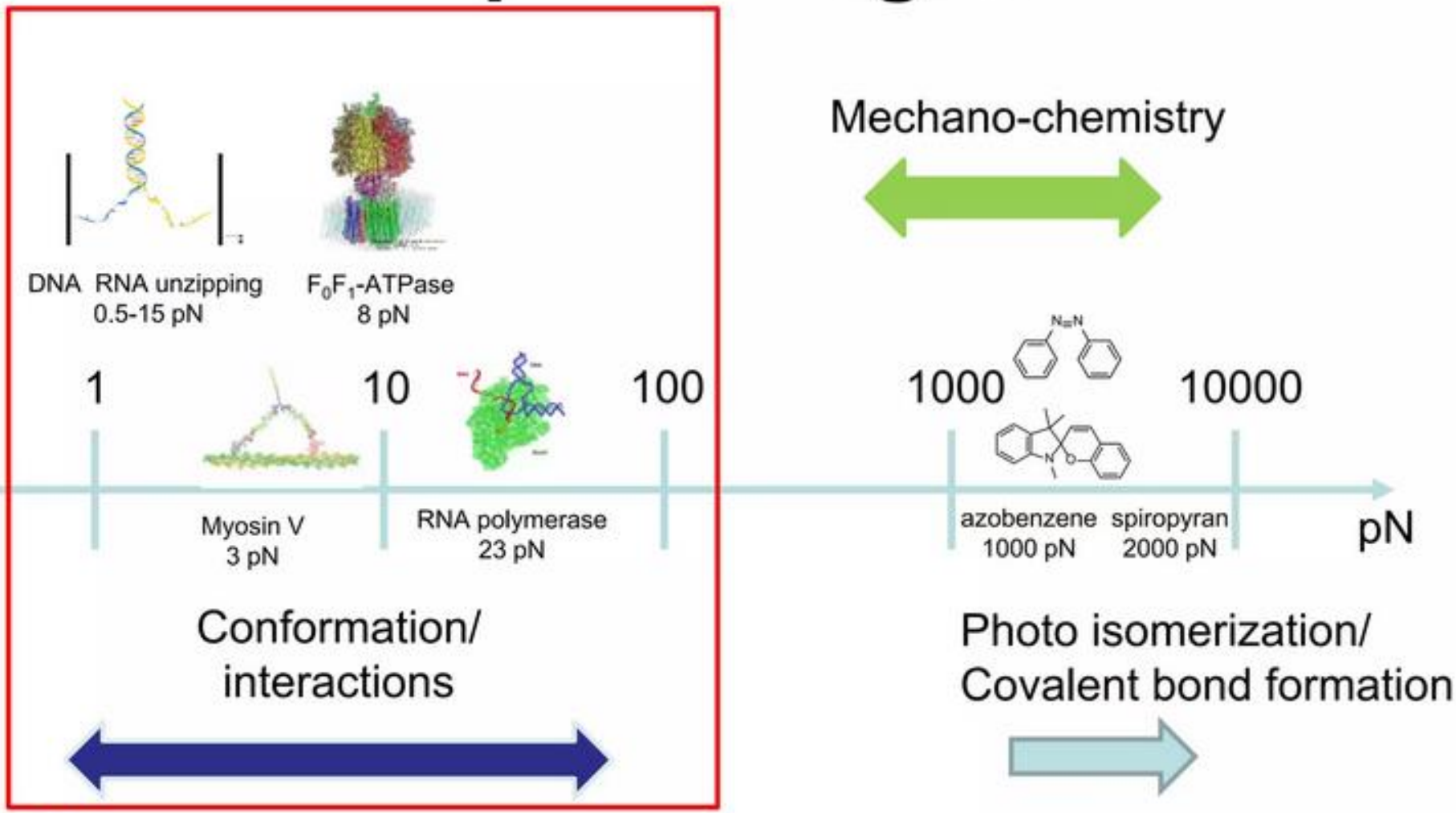
Estimated energy (integral of π-A curve)

≅ 1 kcal/mol

Thermal fluctuation occurs when energy barrier is under 20 kcal/mol.

Oki, M. *Proc. Jpn. Acad., Ser. B* **2010**, *86*, 867–883.

Langmuir technique can cover unexplored region

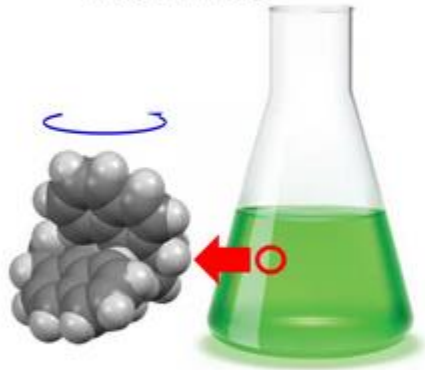


Well-controlled at the air-water interface

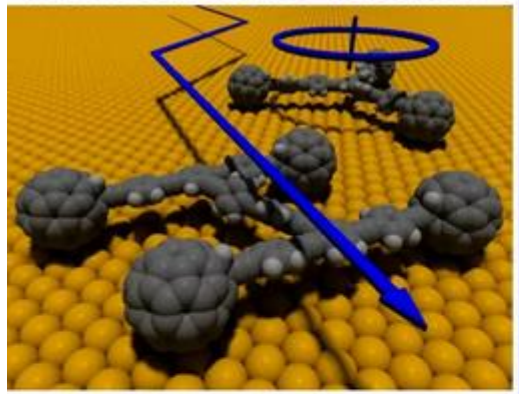
First Demonstration

Catch and Release a molecule by our hand motions

Invisible molecular machines



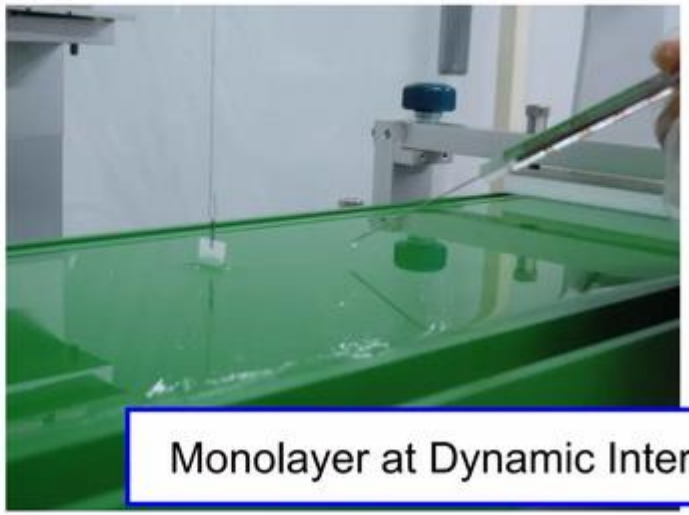
Nanocar under vacuum



Useless ???

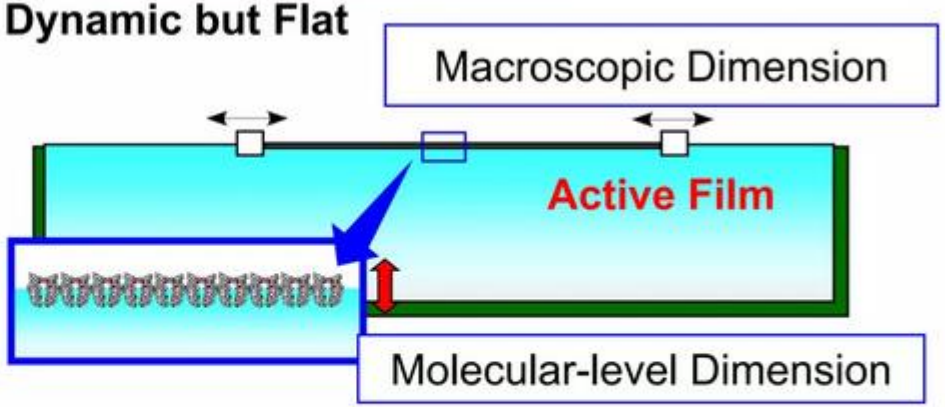
Molecular machines have got Nobel Prize!

Molecular Machine at Dynamic Interface



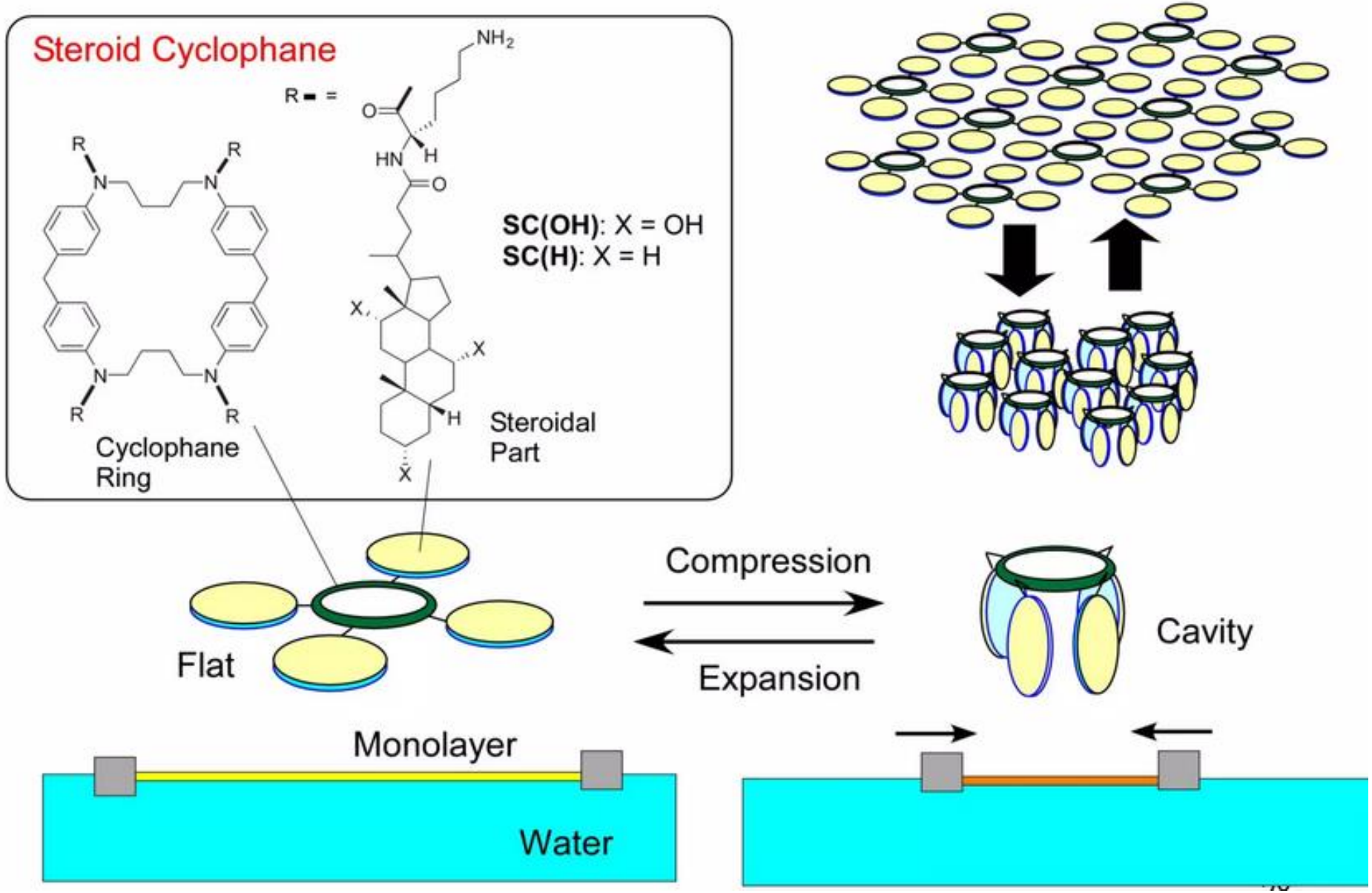
Monolayer at Dynamic Interface

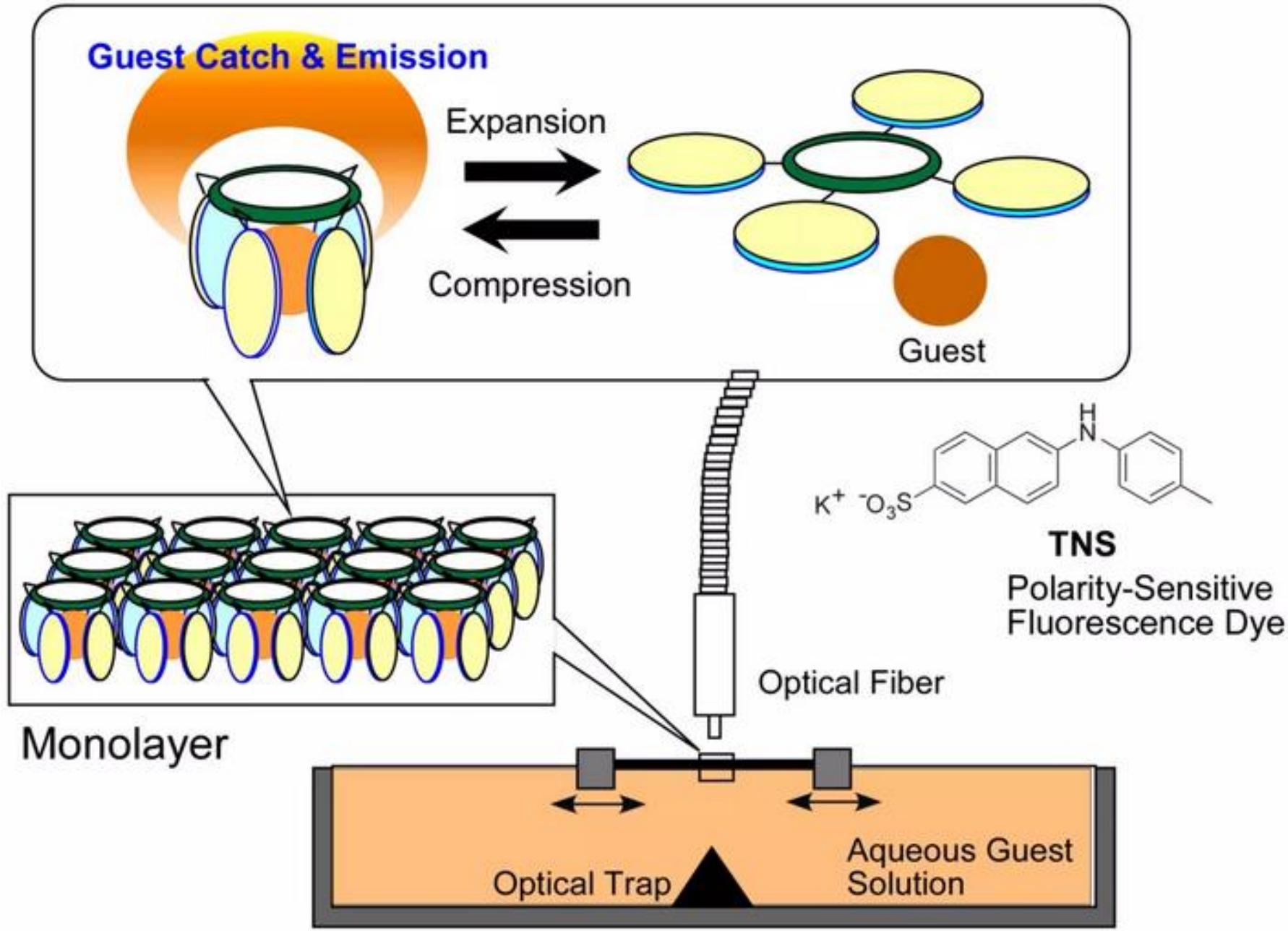
Dynamic but Flat

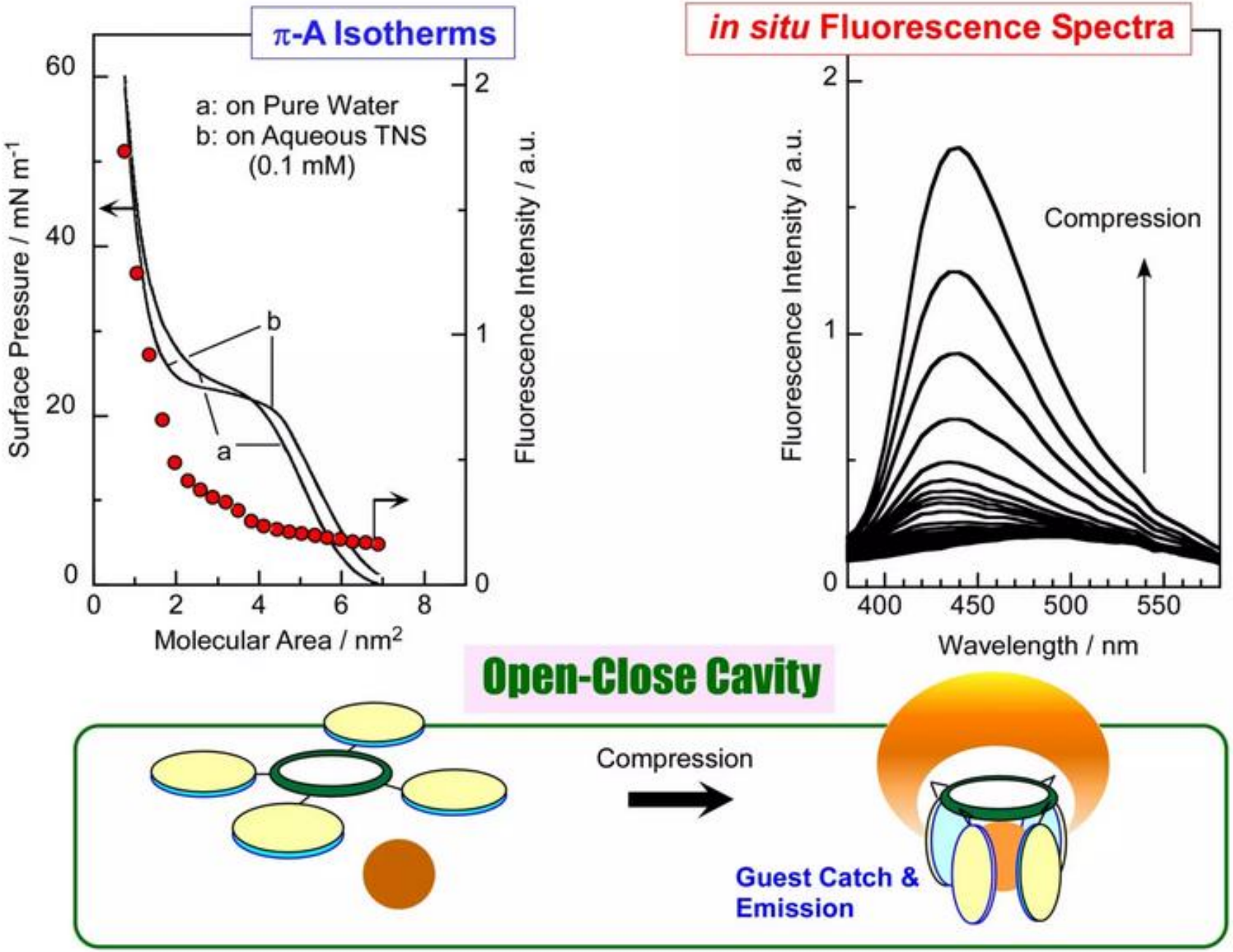


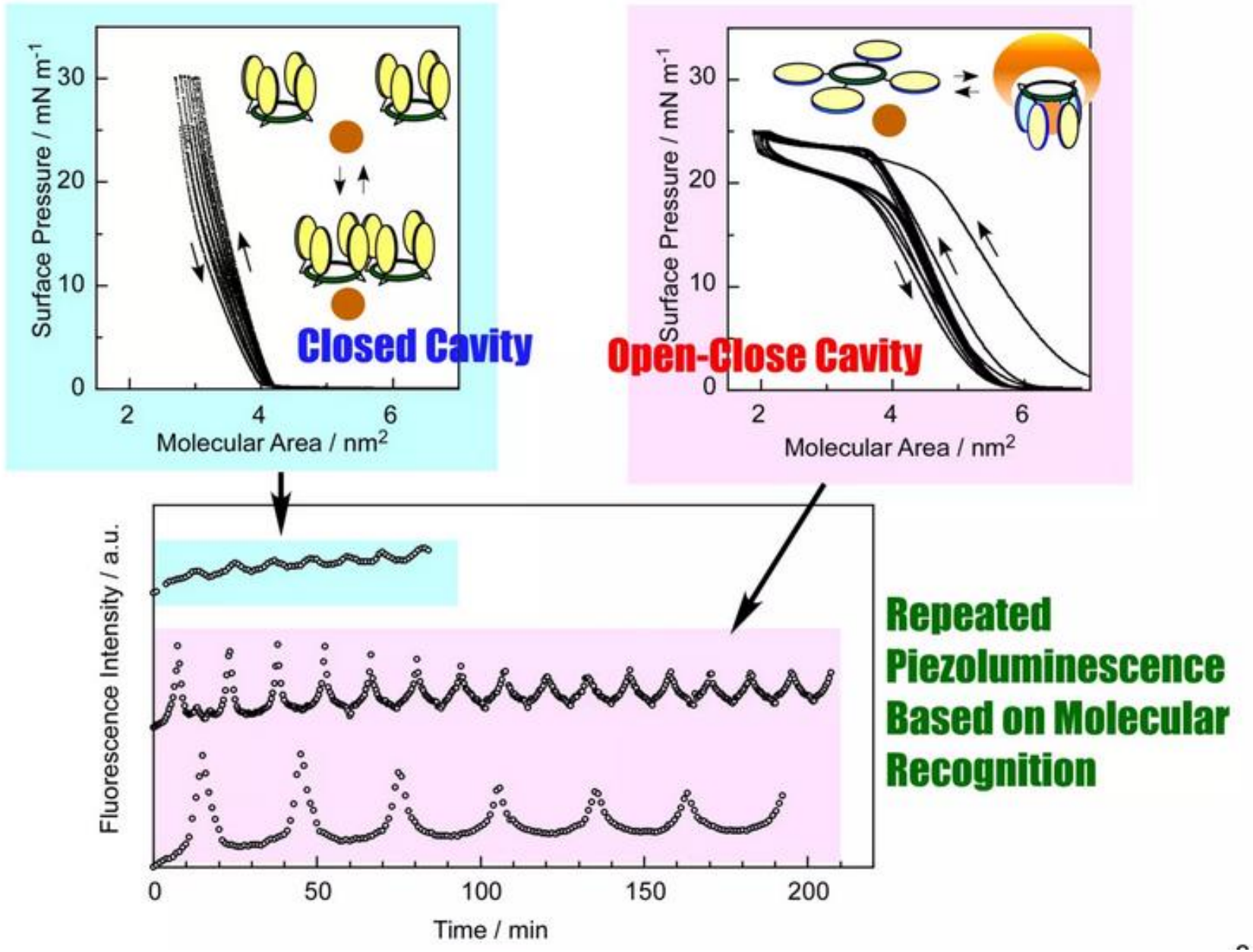
Environment with both molecular and macroscopic characteristics!

Dynamic Function of Molecular Pattern at Air-Water Interface




Guest Catch & Emission
Expansion
Compression
Guest
Monolayer
Optical Fiber
TNS
Polarity-Sensitive
Fluorescence Dye
Optical Trap
Aqueous Guest
Solution


π-A Isotherms
a: on Pure Water
b: on Aqueous TNS
(0.1 mM)
Surface Pressure / mN m-1
Fluorescence Intensity / a.u.
Molecular Area / nm2
a
b
0
20
40
60
0
1
2
0
2
4
6
8
in situ Fluorescence Spectra
Fluorescence Intensity / a.u.
Wavelength / nm
Compression
0
1
2
400
450
500
550
Open-Close Cavity
Compression
Guest Catch &
Emission

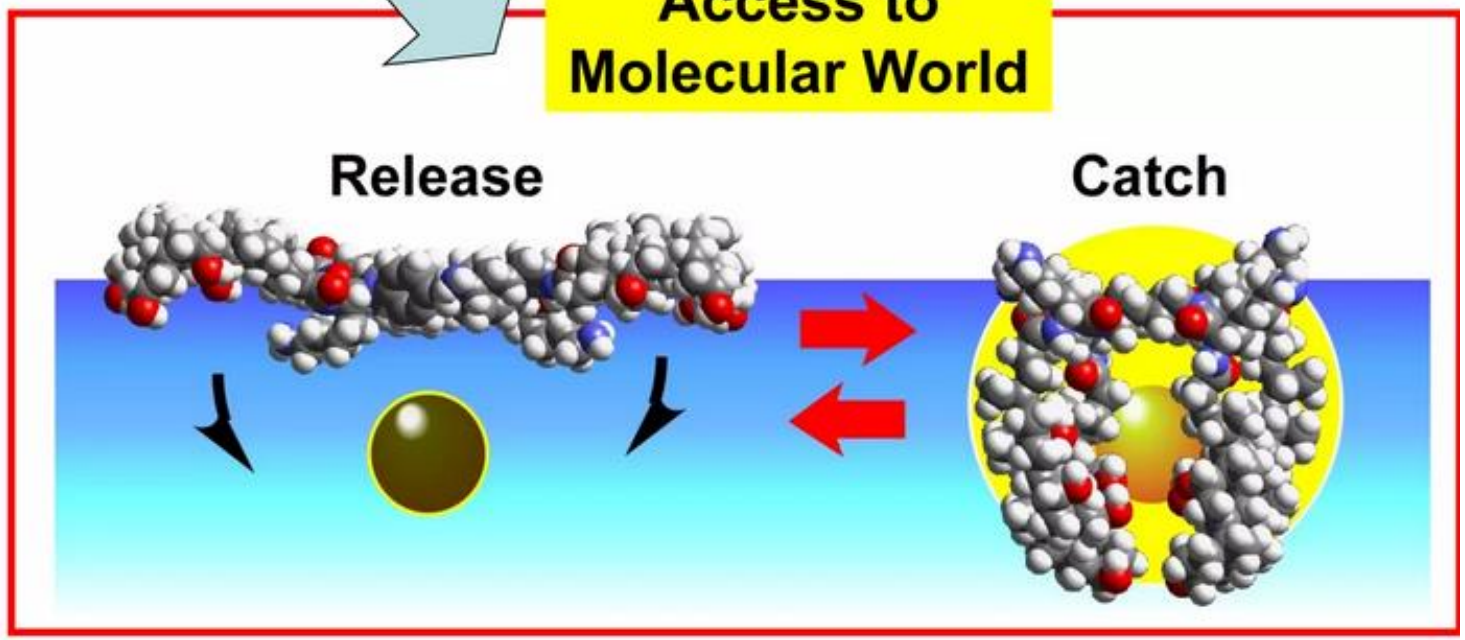

Surface Pressure / mN m-1
Molecular Area / nm2
Closed Cavity
Open-Close Cavity
Fluorescence Intensity / a.u.
Time / min
Repeated Piezoluminescence Based on Molecular Recognition

Piezoluminescence Definition

Luminescence produced by the action of pressure on certain solids

Hand-Operating Nanotechnolog
Molecular Machine at Interface

Connection between molecular (nano) worlc and real (visible) worlc



Second Demonstration

Tuning of Receptors

New Mode of Molecular Recognition

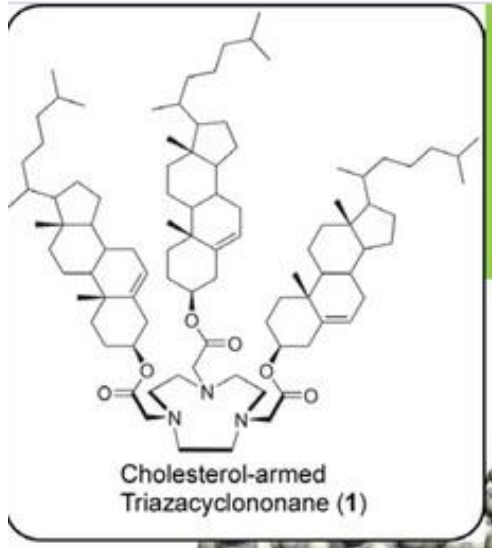
Second Demonstration

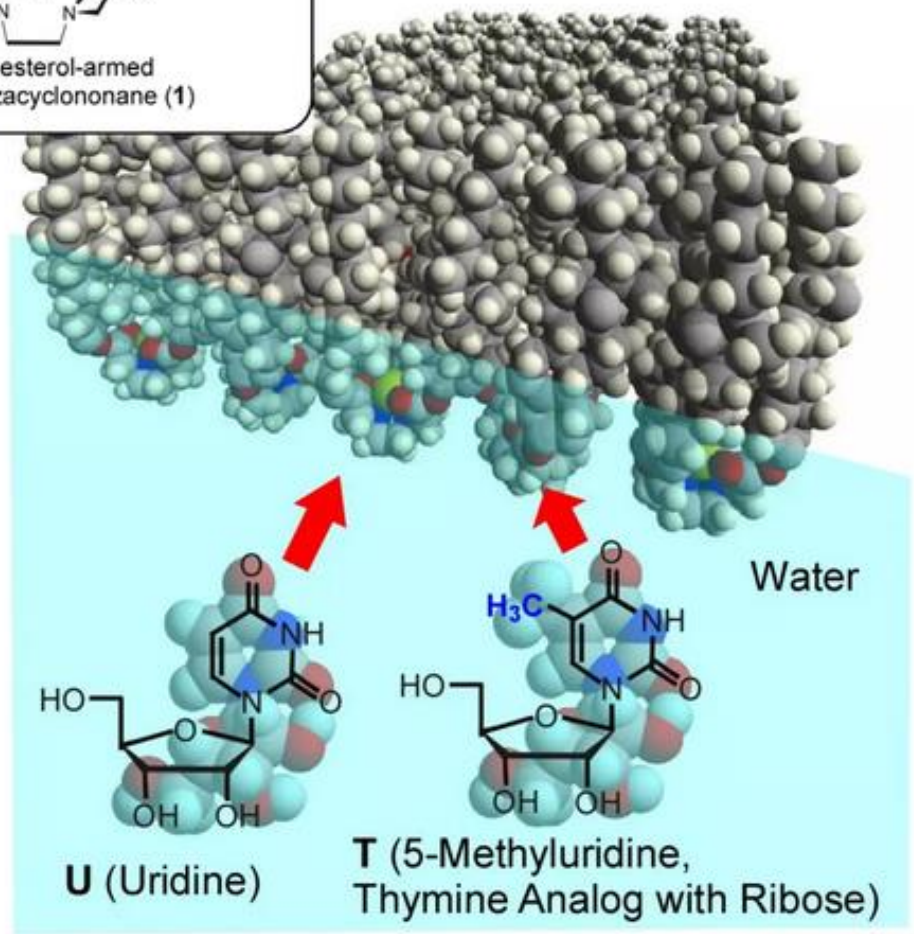
Finer Tuning of Receptors

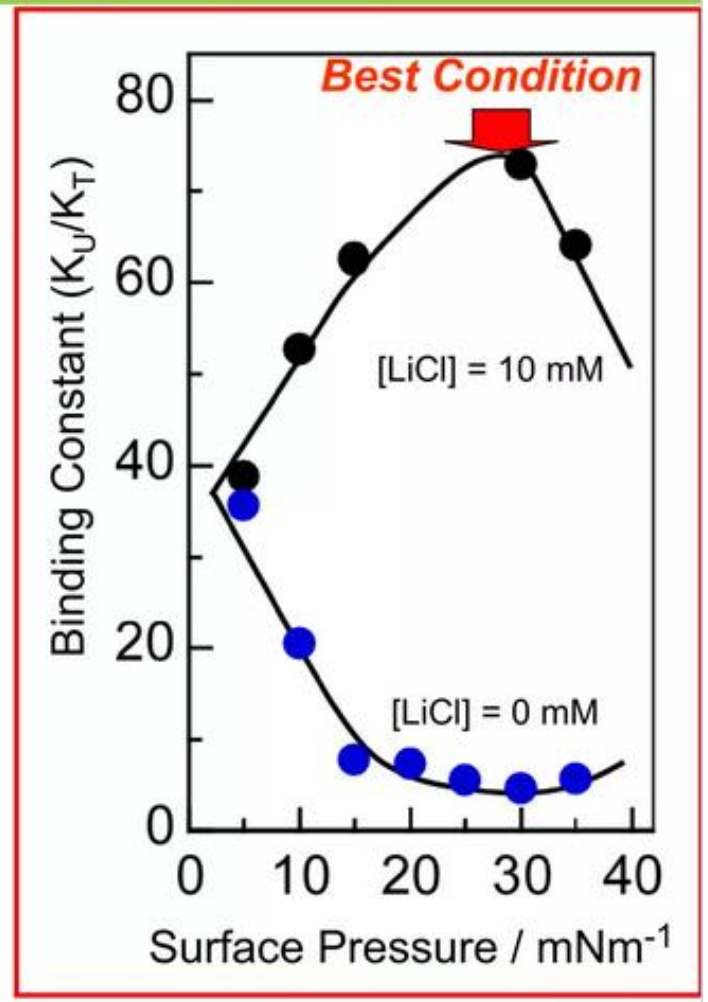
Precise Discrimination
of Biomolecules
by Mechanical Motions

New Mode of Molecular Recognition
Tuning Receptor

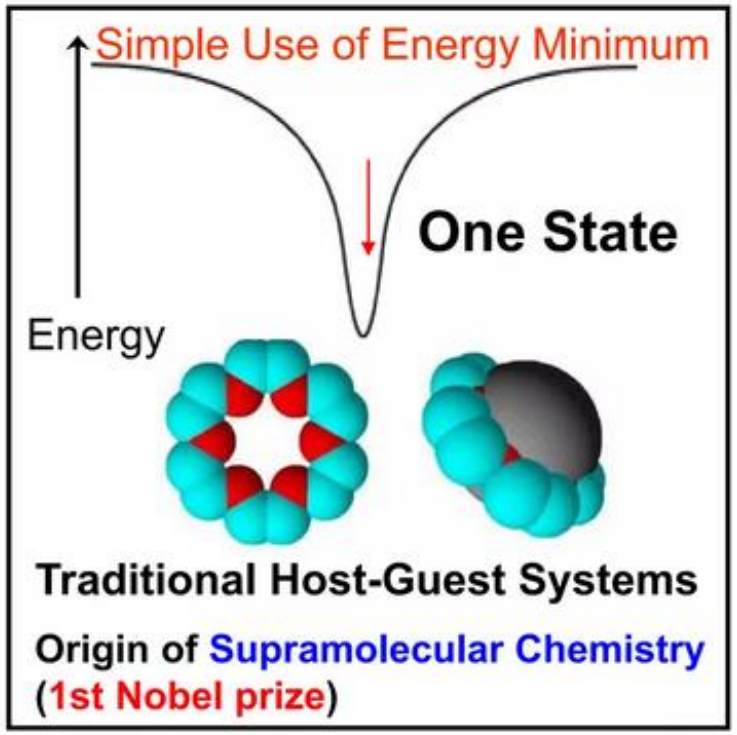
Mechanical Tuning of Molecular Machines for Nucleoside Recognition at the Air-Water Interface

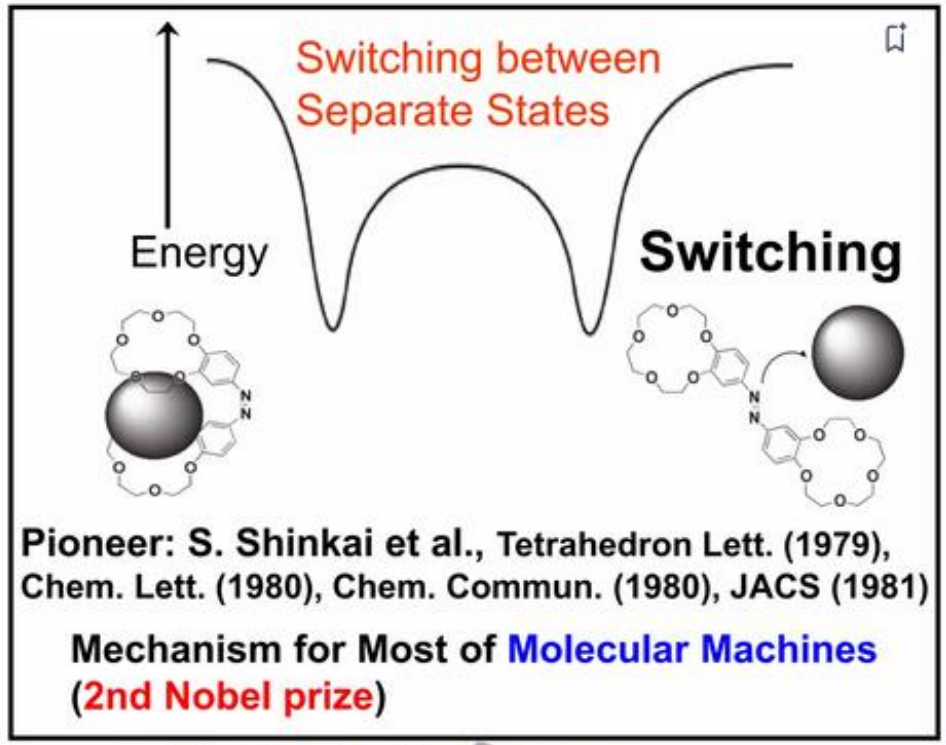




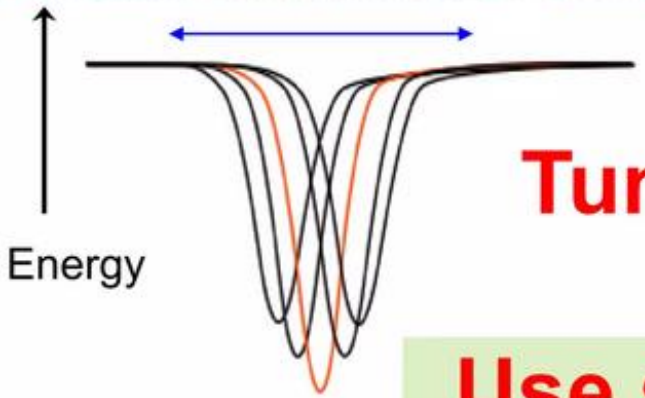


J. Am. Chem. Soc., 132, 12868 (2010).





Continuous Modulation to Find
Best Solution from Numerous Candidates



Tuning

Mechanical Tuning of
Conformation of Host Molecule

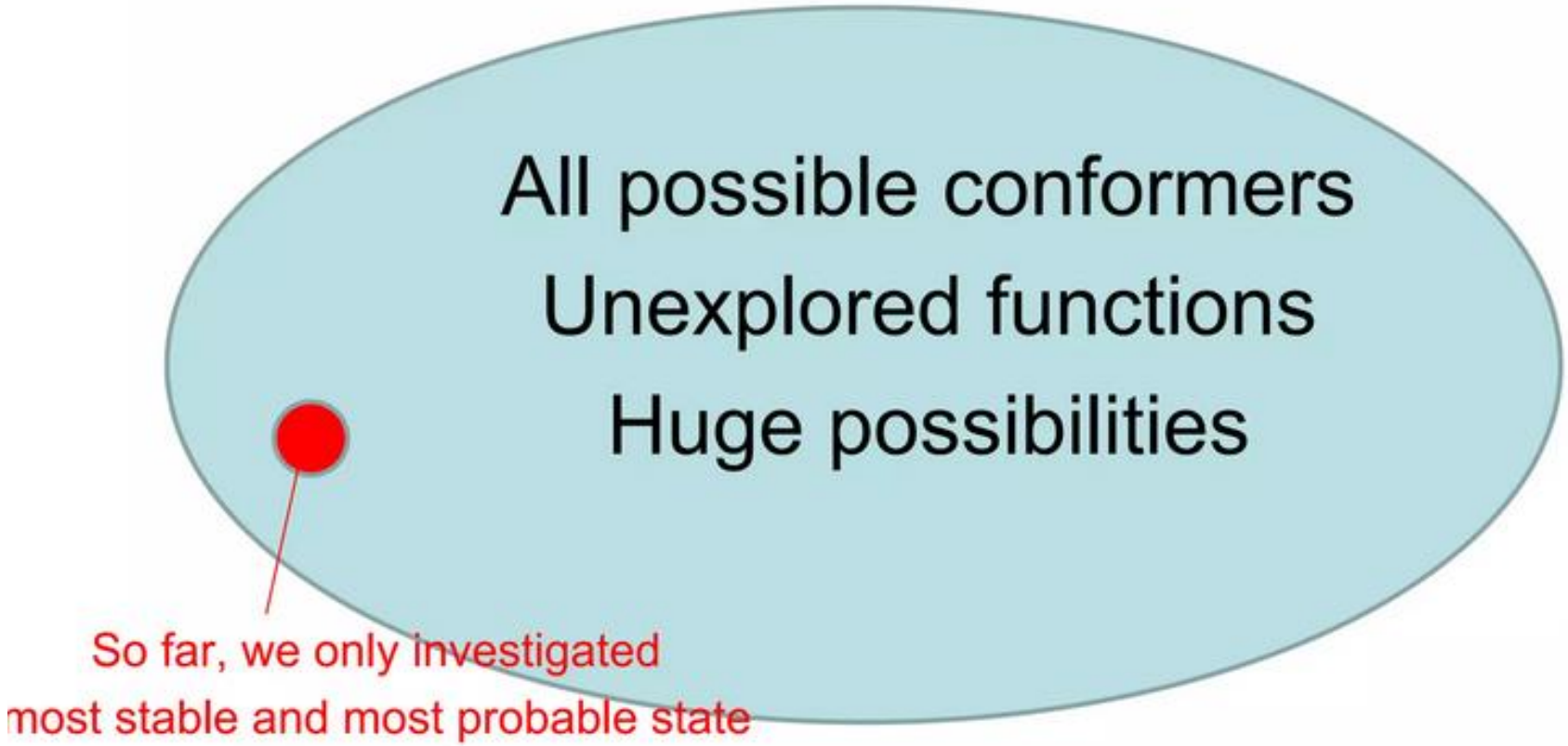
Hand-Operation

Use soft materials softly.

New concept may come

Functional Conformer Science

Tuning molecules toward best function
at outside of simple equilibrium



How is energy efficiency of

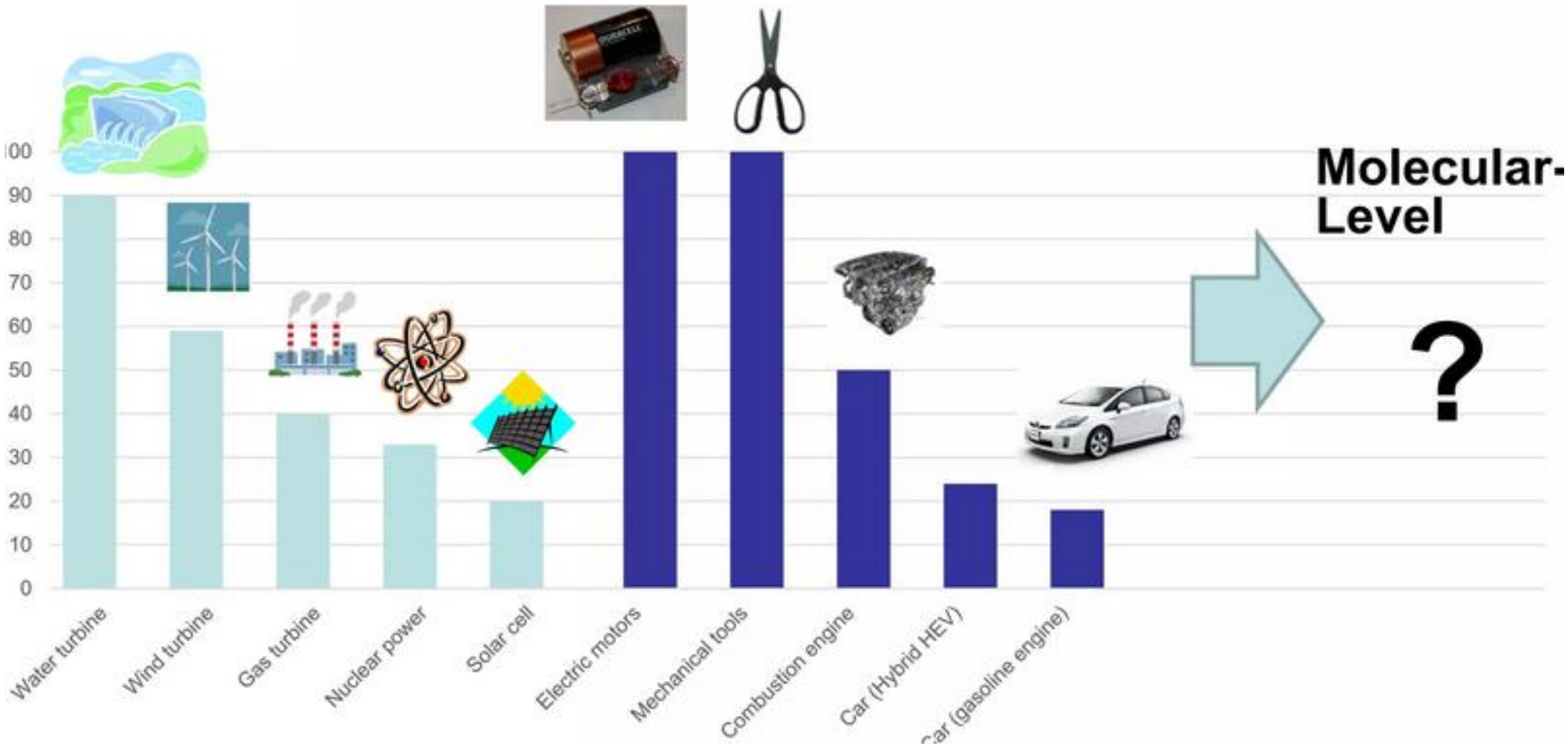
Hand-Manipulation of molecule at Interface

Exploration with model system

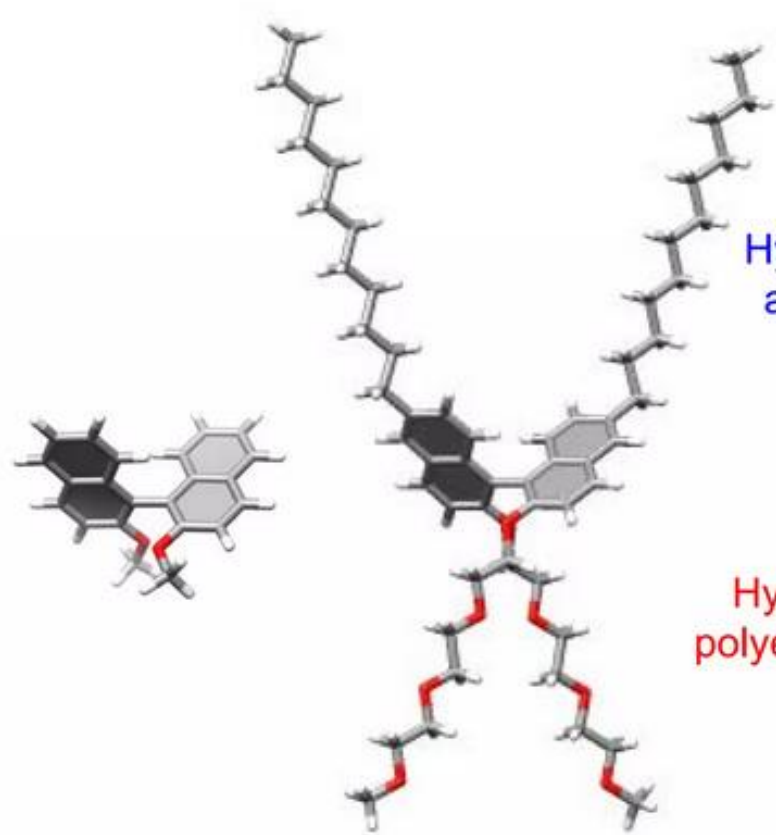
Energy efficiency:

High efficiency with simple direct contact of force and motion.

Can we extend this assumption to molecular-level?

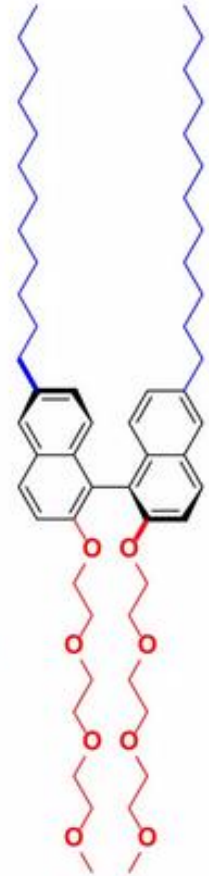


Simple mechanical molecular machine



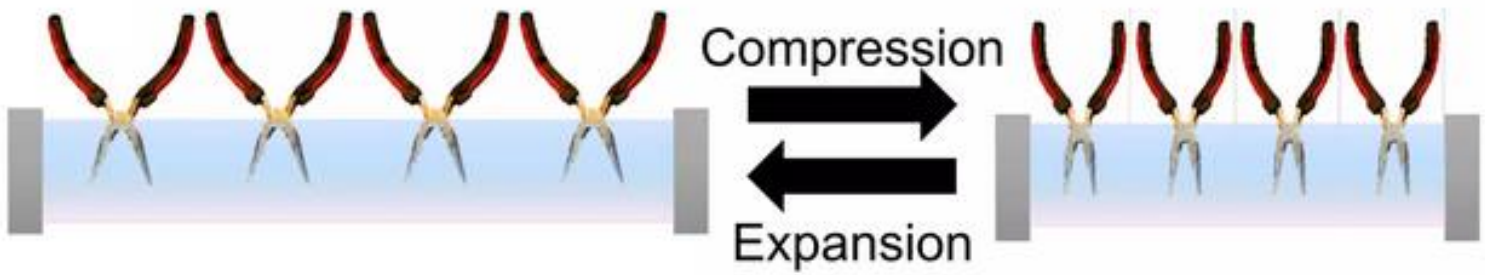
Hydrophobic
alkyl chain

Hydrophilic
polyether chain



Dr. Waka Nakanishi

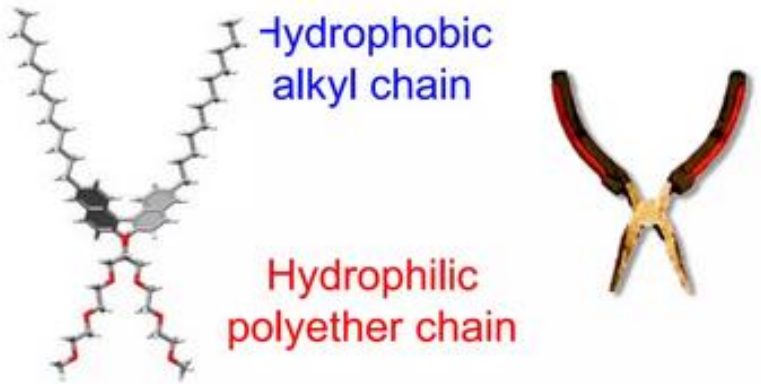
Recent Work: Mechanochemical Control of Simple Molecular Machine, a Nano-Pliers

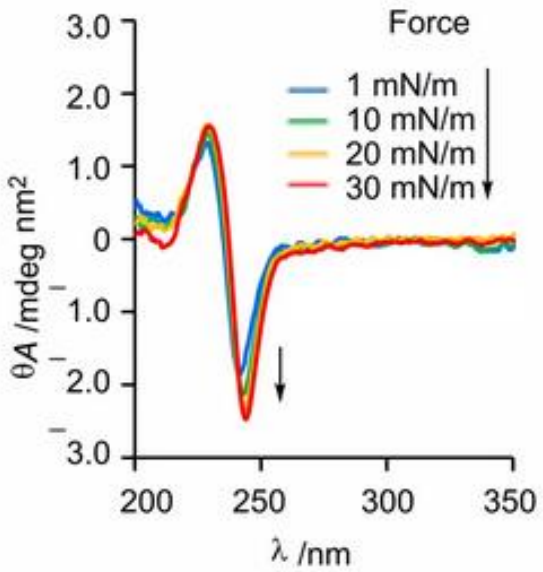


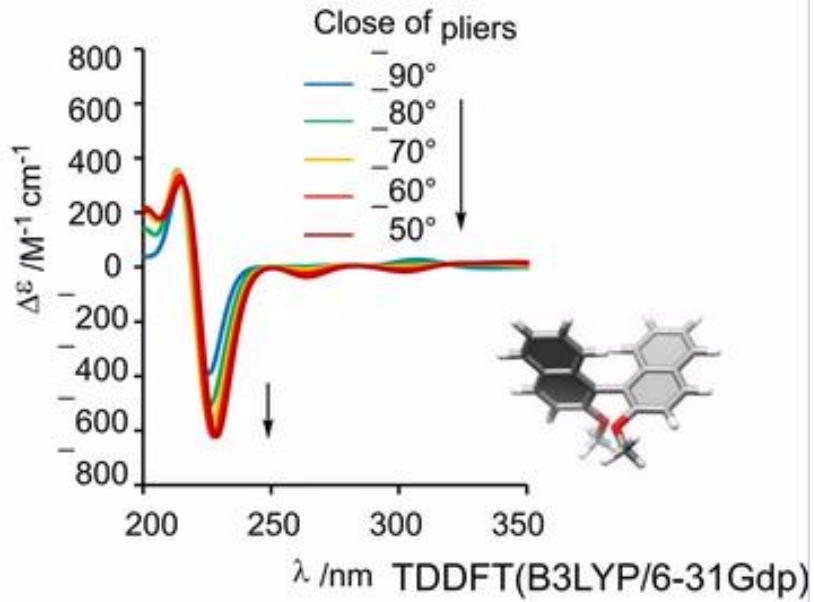
Angew. Chem., Int. Ed., 54, 8988 (2015).

Gradual and reversible tuning of the torsion angle of an amphiphilic chiral binaphthyl, from −90° to −80°, was achieved by application of a mechanical force to its molecular monolayer at the air–water interface. This 2D interface was an ideal location for mechanochemistry for molecular tuning and its experimental and theoretical analysis, since this lowered dimension enables high orientation of molecules and large variation in the area. A small mechanical energy (<1 kcal mol^{-1}) was applied to the monolayer, causing a large variation (>50 %) in the area of the monolayer and modification of binaphthyl conformation. Single-molecule simulations revealed that mechanical energy was converted proportionally to torsional energy. Molecular dynamics simulations of the monolayer indicated that the global average torsion angle of a monolayer was gradually shifted.

Theory & Experiments Revealed Motion of a Molecule





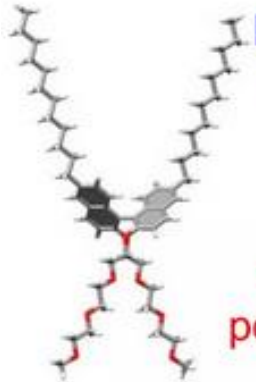


Experimental data from transferred monolayer

Simulations of single molecule

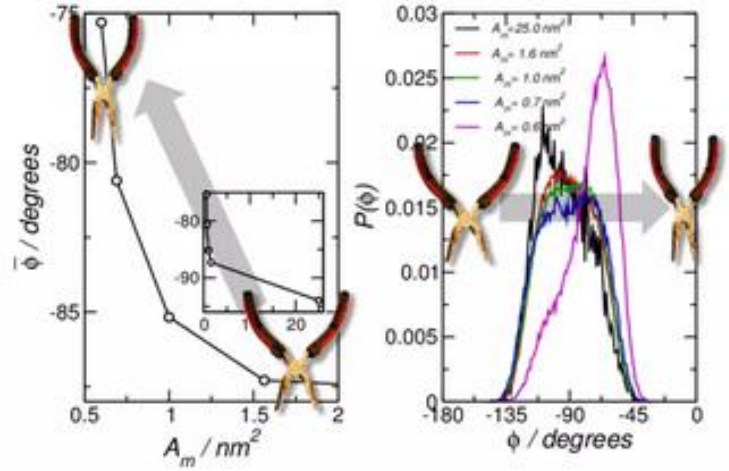
MD Simulations

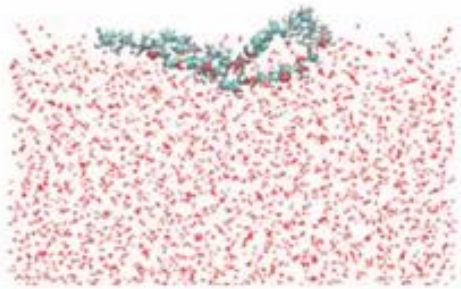
Revealed Structures of Molecules in an Assembly



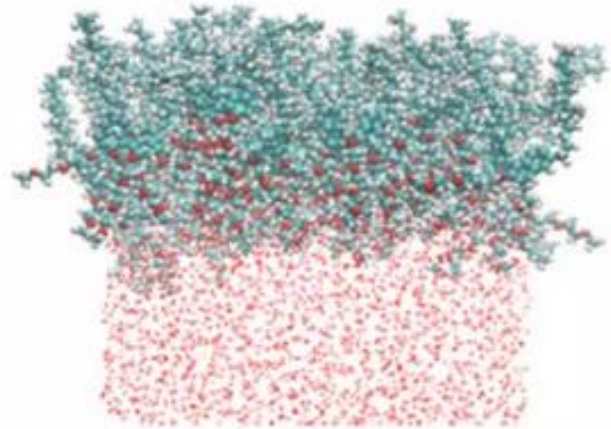
Hydrophobic
alkyl chain

Hydrophilic
polyether chain





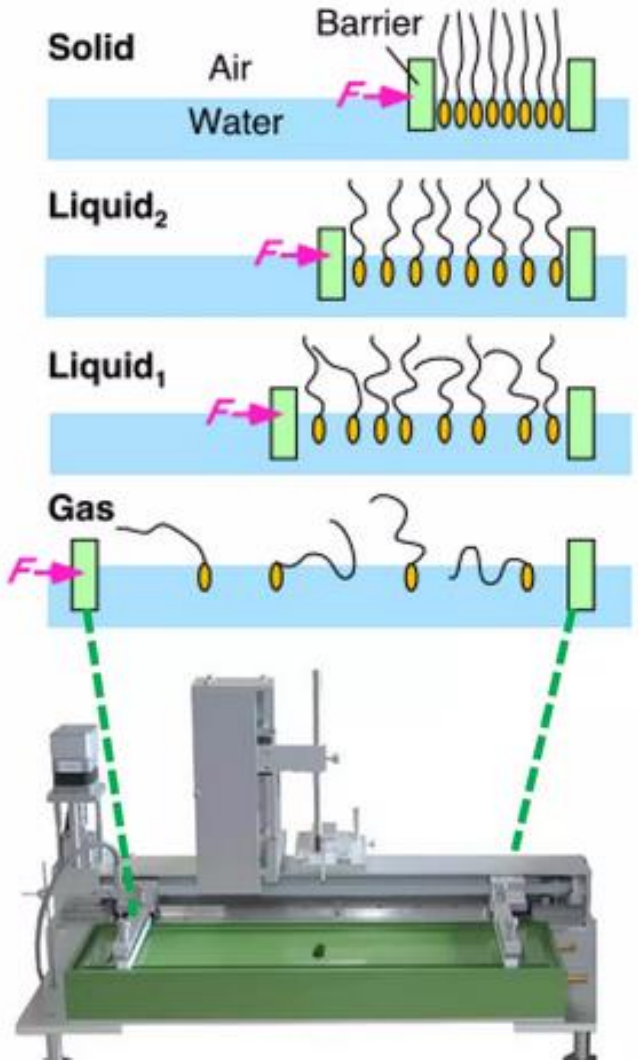
Expanded

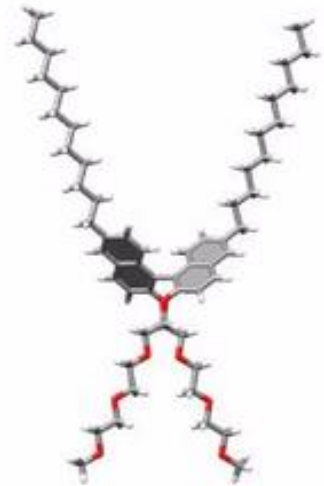


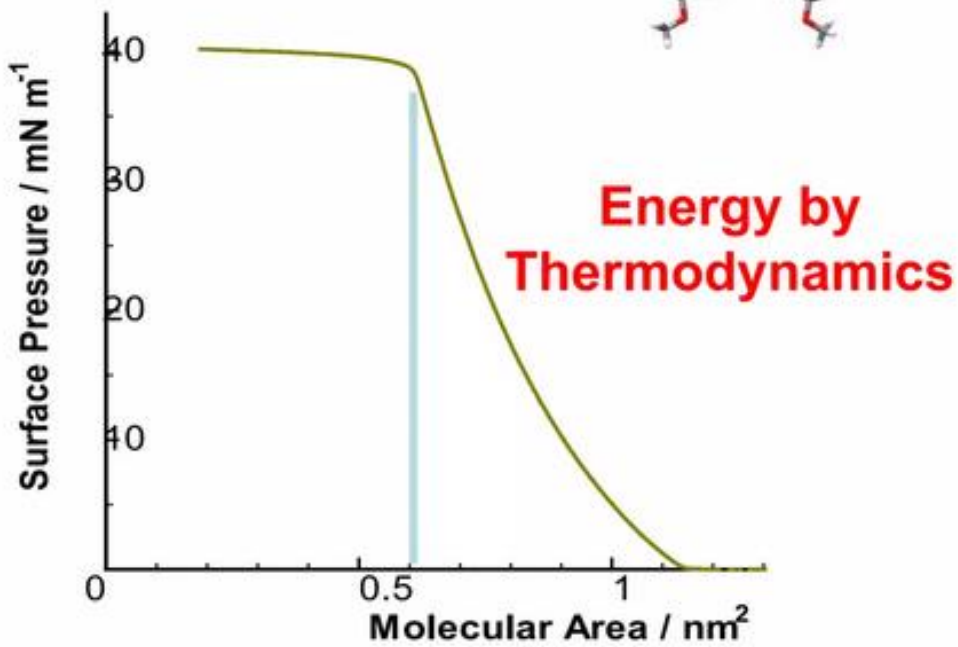
Compressed

Dr. D. Cheung, National University of Ireland Galway

Energy given at the air-water interface

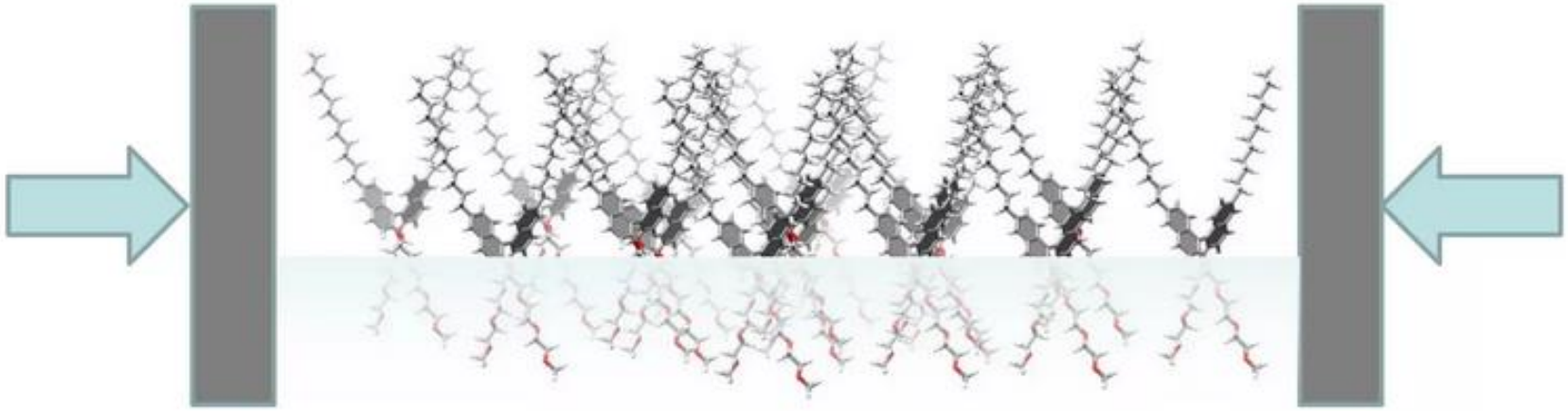


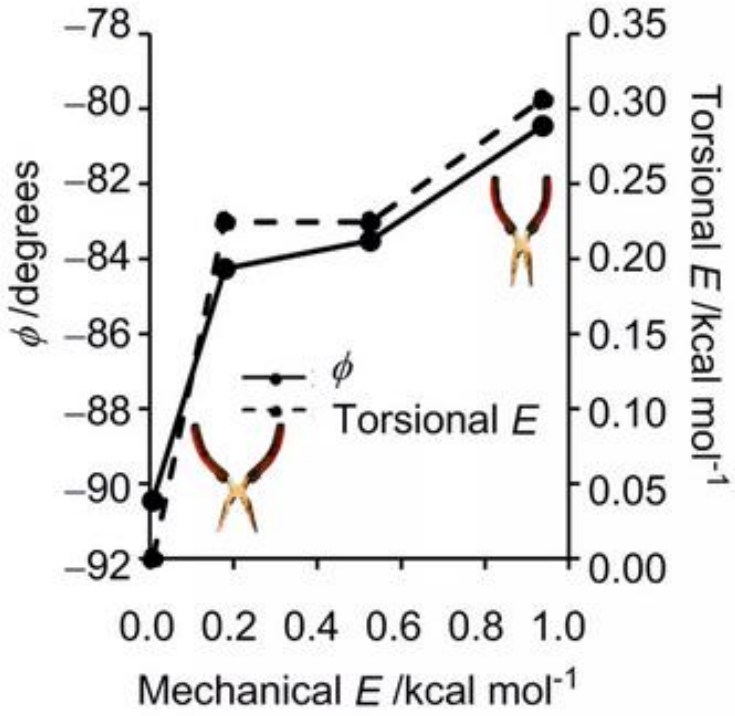




Energy by Thermodynamics

Characteristic Properties at the Air-Water Interface 3



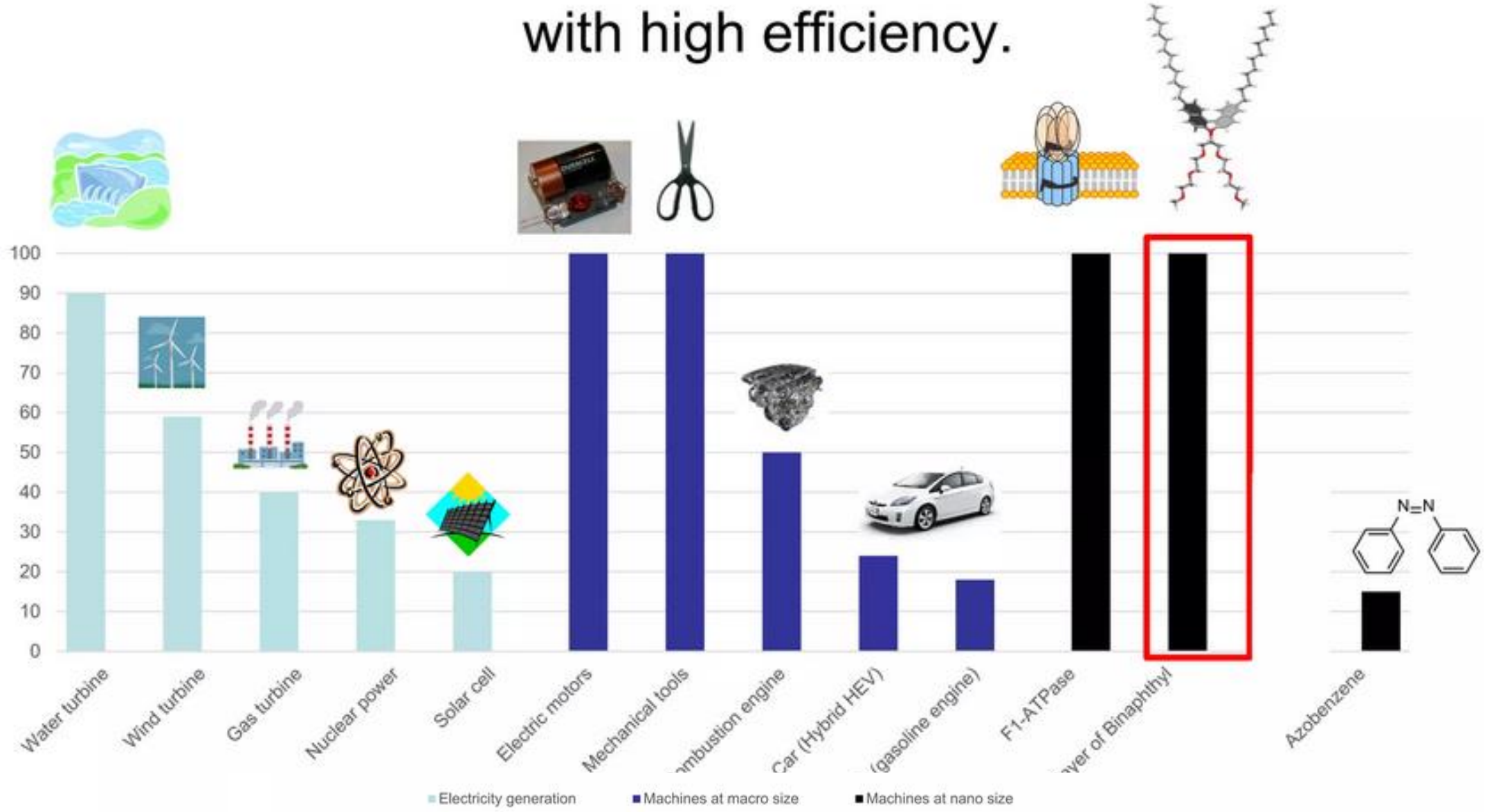


Estimated ϕ and obtained torsional energies v.s. applied mechanical force

molecule

Control of conformational change of molecules and proteins is possible.
Efficient energy conversion is expected.

Mechanical molecular machine works with high efficiency.



Direct mechanical driving is best method.

Unified Understanding
on
Relation between
Force and Motion
from MACRO to NANO

Comparison of Forces [N]

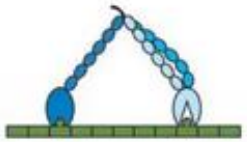
Macroscopic

Car
45 kN

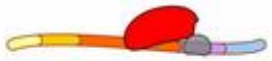
Human
8 kN

Beetle
10 N

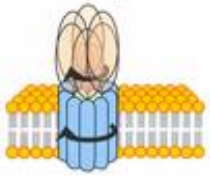
Biology



Myosin V
3 pN

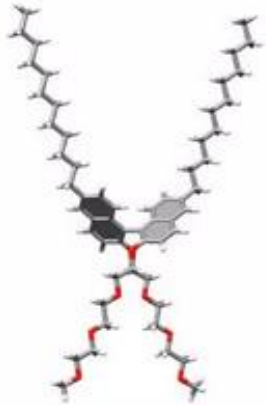


RNA polymerase
23 pN

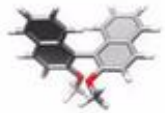


F_0F_1-ATPase
8 pN

Molecular Machine



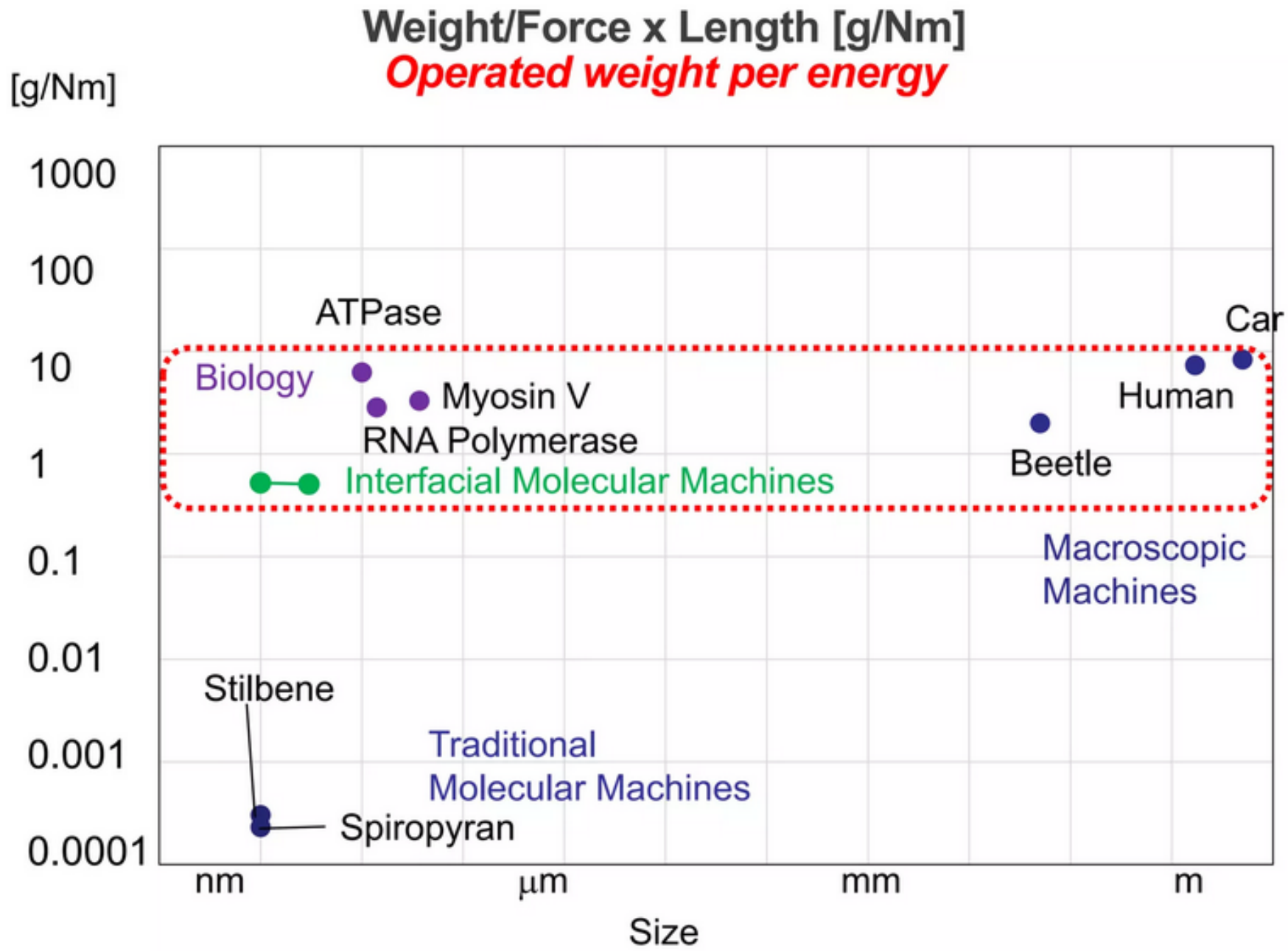
Binaphthyl
1 pN



Binaphthyl
+Lipid Matrix
1 pN

Interfacial Mechanical Molecular Machine

Size 1 m 10 nm 1 nm


Weight/Force x Length [g/Nm]
Operated weight per energy
[g/Nm]
1000
100
10
1
0.1
0.01
0.001
0.0001
ATPase
Car
Biology
Myosin V
Human
RNA Polymerase
Beetle
Interfacial Molecular Machines
Macroscopic
Machines
Stilbene
Traditional
Molecular Machines
Spiropyran
nm
μm
mm
m
Size