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Preview

# Cages Driven Away from Equilibrium Binding by Electric Fields

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**Molecules and materials that are pushed away from equilibrium can produce unique dynamic and adaptive properties that cannot be attained when they are at rest. In this issue of *Chem*, Cockroft and co-workers study the transient binding and debinding of coordination cages to the nanopocket of α-hemolysin and show that the binding and debinding events are driven out of equilibrium by an external electric field. Strong applied fields can invert the cage-nanopore binding selectivities and can enhance enantio-inversion to enrich one chiral form of the cage over the other.**

All living systems exist far from equilibrium, demanding a continuous influx of matter and/or energy for survival. For example, cytoskeletal microtubules, which can act as tracks for kinesin and dynein biomotors,[1] form by self-assembly of tubulin dimers but only when mediated by GTP (guanosine triphosphate).[1] Hydrolysis of GTP to GDP (guanosine diphosphate) and inorganic phosphate leads to the disassembly of microtubules, resulting in the collapse of its filament structure. Under physiological conditions, GTP-fueled polymerization and hydrolysis-driven depolymerization occur simultaneously such that their relative rates dictate net elongation or shrinking of the microtubules. This dynamic property arises when the system is not at equilibrium and is a ubiquitous feature of biology.

Over the years, our growing understanding of natural and artificial systems sitting at thermodynamic equilibrium has allowed us to design new functional materials with high precision and predictability. However, the number of designed molecular systems displaying properties that emerge only when pushed away from equilibrium by the influx of energy and/or matter and that are inaccessible to equilibrium states is few and far between.[2] Some of the early examples have only been realized in recent years. For example, inspired by biological microtubules, van Esch, Eelkema, and co-workers reported the transient growth of one-dimensional supramolecular fibers[3] upon consumption of chemical fuels and its sudden collapse upon removal of the fuel.

In this issue of *Chem*, Cockroft and co-workers describe a far-from-equilibrium system[4] involving one of the most elementary systems in supramolecular chemistry—that of the host-guest complex. One of the key features of this study is the ability to measure the time-dependent cage-nanopore binding events as blockages of the *cis* side of α-hemolysin[5] by using an external electric field. In so doing, the authors measure the on-off rates for binding as well as the equilibrium constants and thus use the same platform to drive the system away from equilibrium, verify the shift in populations, and identify new behaviors.

The coordination cages used to block the *cis* opening of the nanopore are water-soluble $M_4L_6$ coordination cages (M, metal ion; L, ligand) bearing either positive or negative charges of different magnitudes (12−, 8−, 4−, 12+, 12+, 8+). The authors introduce the well-known α-hemolysin[5] as a nanopore into a lipid bilayer. Under an external transmembrane voltage applied across the bilayer, a characteristic current of ions flows through the nanopore of α-hemolysin. In this field-perturbed environment, the addition of tetrahedral cages on the *cis* side leads to blockage of the *cis* entrance at the single-molecule level. These binding events result in a temporary and discrete fluctuation of the transmembrane current. Following the ion current signatures of these binding events allows the authors to obtain the on-off rates ($k_{on}$-$k_{off}$) of cages with nanopores under external potential. As one might expect, positive and negative transmembrane potentials are used for measuring the binding of negatively and positively charged cages, respectively.

Under an external electric field, the equilibrium constant, which is the ratio of the association and dissociation rates of cages with a nanopore ($k_{on}/k_{off} = K_a$), increases linearly with increased applied electric potential, as expected. The extrapolation of these data to zero applied potential provides the intrinsic value of the association constant. These extrapolated values suggest little to no binding of cages with the nanopore under non-perturbed conditions.

The transient binding of cages with the nanopore also depends on the local electrostatic interactions between cages and the positively charged lysine residues at the *cis* entrance of

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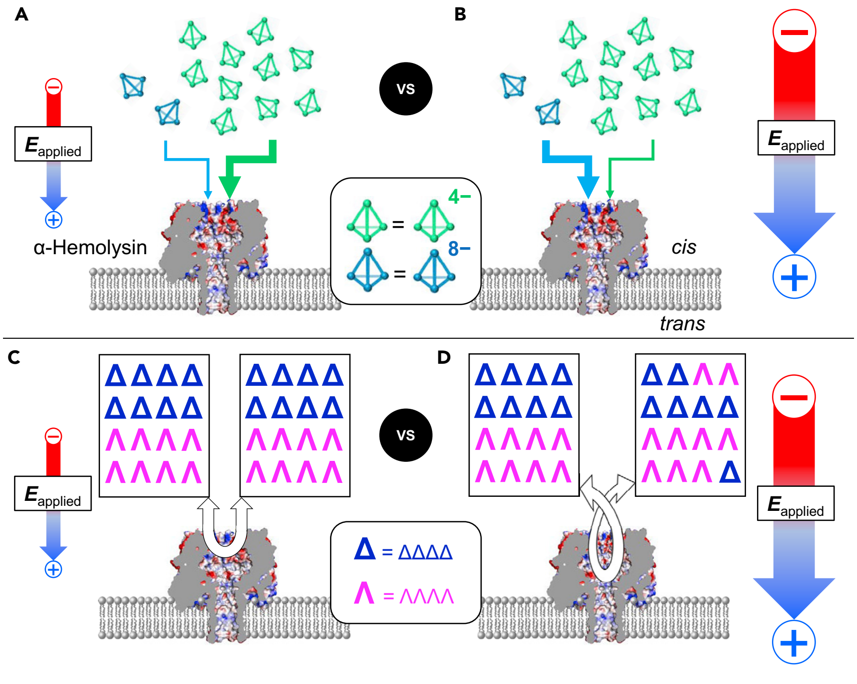

**Figure 1. Out-of-Equilibrium Binding of Coordination Cages to Nanopores**

(A and B) Binding of a 4− tetrahedral cage to the *cis* side of the chiral α-hemolysin nanopore under low electric field follows Le Chatelier's principle (A), but use of a larger applied field inverts the binding selectivity to favor the 8− cage present at lower concentrations (B).

(C and D) Both enantiomers of a $Ge^{4+}$ tetrahedral cage (8−) bind and debind to the nanopore without changing their stereochemistry (C) until the electric field is increased to display more frequent enantio-inversions of the ΔΔΔΔ stereoisomer (D).

α-hemolysin. As a result, the dissociation rate of negative cages from the nanopore (when positive applied potentials are used) is lower than that of positive cages (when negative applied potentials are used). Whereas the external electric field dictates the transient binding of cages, the local electrostatic interaction governs the dissociation of cages from the nanopores. This observation adds to the emerging interest in understanding how local electrostatics control elementary binding events.[6–8]

The electric field can be used to turn on different behaviors. First, it is found to invert the selectivity of binding between the cages and the nanopore (Figures 1A and 1B). The competitive binding of the nanopore with 8− and 4− cages is tilted in favor of the 4− cages with a 150-fold excess. At low voltage (+50 mV), Le Chatelier's principle is followed such that the binding of 4− cages is preferred over that of 8− cages. However, at higher potential (+150 mV), the binding selectivity is reversed, i.e., the nanopore binds the 8− cages preferentially. This effect arises from the higher charge density of the 8− cages, which debind less frequently than the 4− cage under the larger external field. This field drives the population of the bound states away from equilibrium.

All the tetrahedral cages exist as a racemic mixture of two enantiomers, where all four metal centers are either ΔΔΔΔ or ΛΛΛΛ. In a previous publication,[9] Lusby, Cockroft, and co-workers exploited the intrinsic chirality of the nanopore to discriminate the chirality of tetrahedral cages under an applied electric potential. Detection of the cages with different chirality benefits from the $Ga^{3+}$-based cages' retention of their chiralities at the applied voltage.

In this issue of *Chem*,[4] the authors observe an interesting electric-field-driven enantio-inversion of 8− tetrahedral cages based on $Ge^{4+}$ metal centers (Figures 1C and 1D). This inversion occurs when the cages are bound inside the nanopore but only at an applied potential of +120 mV. At lower voltages, e.g., +80 mV, enantio-inversion events are very rare. The enantio-inversion of cages induced by the applied potential proceeds through meta-stable states, as seen in the transient current measurements. Interestingly, the ΔΔΔΔ cages exhibit more metastable events than the ΛΛΛΛ cages, leading to a net enhancement in ΛΛΛΛ cages. The single-molecule measurements, however, also verify the less-frequent enantio-inversion in the other direction from ΛΛΛΛ to ΔΔΔΔ. The chiral nanopocket of hemolysin most likely leads to this selectivity.

In summary, this study illustrates the influence of an external electric field on the binding and debinding of charged cages with the nanopore of α-hemolysin. Given that the sizes and charges of the cages are synthetically modifiable, the authors understand and then control the transient behavior of the cages. The magnitude of the electric field is used to create out-of-equilibrium conditions to control the selective capture and release of cages and their stereo-inversion. In the future, it would be interesting to investigate whether it would be possible to exploit the interior of a cage, which has been well explored to capture a variety of guests,[10] to get unique functions under these non-equilibrium conditions: is it possible to selectively deliver a neutral guest residing inside a tetrahedral cage to the *trans* side of α-hemolysin? We envision that surprising new properties will continue to emerge when we learn how to control and study such "living" molecular systems.

## ACKNOWLEDGMENTS

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### Preview

# Protein Mass Spectrometry: Structural Characterization and Clinical Diagnosis

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**In the early 1980s, the development of two ionization techniques—electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI)—opened the door for the analysis of intact large biomolecules via mass spectrometry (MS). The power of modern protein MS is highlighted by two independent reports in this issue of *Chem*.**

Mass spectrometry (MS) has a long and rich history dating back to 1919 with Aston's first functioning mass spectrometer.[1] As highlighted by McLafferty, MS has always had significant advantages, which he coined the three S's—specificity, sensitivity, and speed—over other analytical techniques.[2] However, early MS instruments were limited to the analysis of small molecules given that the ionization techniques available at the time were just too violent for the analysis of larger, more fragile molecules. This was especially true for large polar organic molecules, such as proteins and other biomolecules. It wasn't until the advent of the so-called "soft" ionization techniques of electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) in the late 1980s that this began to change, and for the first time intact proteins could be gently transformed into gas-phase ions (Figure 1).[3–5] The era of biomolecular MS was ushered in, and the importance of these two techniques was recognized in 2002 with the award of the Noble Prize.

Since then, MS has become an indispensable analytical technique for the biosciences. Perhaps most notable is its place as the cornerstone technology for the field of proteomics, where MALDI and ESI have both been extensively used.[6] Outside the "-omics" fields, MS has become an important technique in structural biology—so-called native ESI techniques allow large non-covalent biomolecular complexes to be directly analyzed[7]—and, together with covalent labeling and crosslinking techniques, is invaluable for reporting on protein structure and dynamics.[8] MALDI has found new important application in the rapidly emerging field of MS imaging.[9] The legacy of these two ionization techniques and their diverse impact on the biological sciences is vast and is exemplified by two independent reports in this issue of *Chem*.[10,11]

In the first of these studies, Aneika Leney, Albert Heck, and co-workers apply state-of-the-art MS techniques to investigate the assembly of the light-harvesting antennas in red algae.[10] Red algae represent some of the oldest lifeforms on the planet, and over billions of years they have evolved

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