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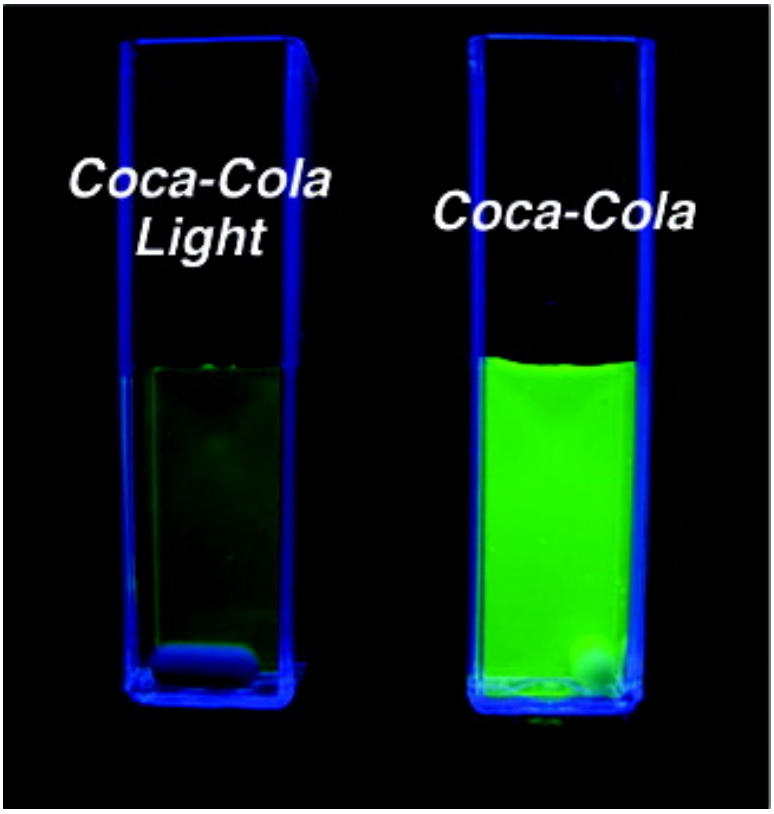

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Sugar Sensing with Synthetic Multifunctional Pores

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Recently, we discovered that chemical reactions can be detected with synthetic multifunctional pores in a universal manner comparable to chromatographic techniques.[1−3] Here, we report the conceptual expansion of this method to sensing applications. This is achieved by using enzymes as variable co-sensors to detect the analyte of choice with synthetic multifunctional pores. To highlight the practical relevance of this concept, sugar sensing in soft drinks with invertases and kinases as co-sensors was selected as an example. The discrimination of ATP and ADP in the "on−off" fashion required for the "naked-eye" detection was of particular interest because it is a challenging topic of general importance[4−6] with diverse applicability.

Previous reports on the discrimination of ATP and ADP with synthetic multifunctional pores have not been very encouraging. For rigid-rod β-barrel pore **1** with internal RH dyads and external LWV triads (Figure 1), the difference between blockage by ATP and ADP was insufficient for on−off naked-eye discrimination (Figure 2Cc vs 2Cd).[2] Recently, however, we succeeded in constructing synthetic multifunctional pore **2** with internal KH dyads without losing key characteristics such as thermodynamic instability that made pore **1** with internal RH dyads the pore of choice in practical sensing applications.[7] Breakthroughs on nucleotide recognition by synthetic β-hairpins in the Waters group[5] suggested that this internal R → K mutation could be the key to practical discrimination of ATP and ADP.[5,6] In the following, we first map out the experimental conditions applicable for naked-eye on−off recognition of ATP over ADP by synthetic multifunctional pore **2** and then corroborate their usefulness as detectors of enzyme activity before focusing on their conceptually innovative use as adaptable, "universal" sensors.

The multifunctional pore **2** was synthesized and characterized as reported previously.[7] The discrimination of ATP and ADP by pore **2** was probed in large unilamellar vesicles composed of egg yolk phosphatidylcholine and loaded with 5(6)-carboxyfluorescein (EYPC-LUVs⊃CF). In the CF assay, pore activity as such is detectable by the disappearance of CF self-quenching during CF efflux, host−guest chemistry as decreasing or increasing pore activity in response to molecular recognition (Figure 2A). Hill analysis of the dose response curve for blockage of synthetic multifunctional pore **2** by ATP gave $IC_{50}^{ATP} = 22\ \mu M$ (Figure 2B●). Comparison with ADP ($IC_{50}^{ADP} = 224\ \mu M$, Figure 2B○) under routine conditions gave a discrimination factor $IC_{50}^{ADP}/IC_{50}^{ATP} = 10$. This value for synthetic multifunctional pore **2** with internal KH dyads was 3 times higher than that reported previously for synthetic multifunctional pore **1** with internal RH dyads.[2]

This significant discrimination of ATP/ADP by synthetic multifunctional pore **2** was tolerant of changes in experimental conditions. The IC_{50}'s for pore blockage by both ATP (●) and ADP (○) decreased with decreasing ionic strength toward saturation in the log−log plot (Figure 3B).[8,9] This trend confirmed ion pairing as the dominant interaction between pore and blockers.[9] The discrimination factor $IC_{50}^{ADP}/IC_{50}^{ATP}$, however, did not depend much on ionic strength (Figure 3B×). Important when working with kinases,[10] the presence of up to ∼100 μM Mg^{2+} was tolerated at both low and intermediate ionic strength without important losses in sensitivity and selectivity. At low vesicle concentrations, discrimination factors $IC_{50}^{ADP}/IC_{50}^{ATP}$ up to 22 could be achieved with reduced pore consumption per assay. However, these less robust conditions were overall less satisfactory and finally abandoned because of the naturally reduced reproducibility.

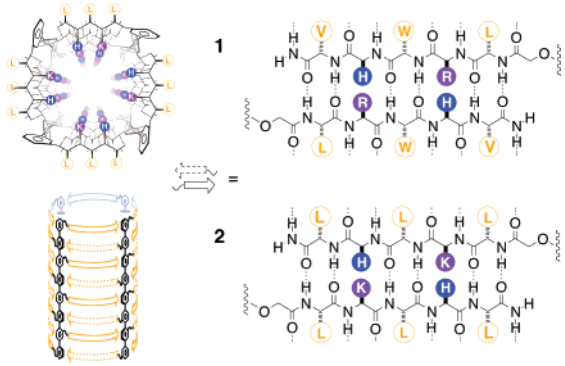

Figure 1. Notional rigid-rod β-barrel pores **1** and **2** with β-sheets as solid (backbone) and dotted lines (hydrogen bonds, top) or as arrows (N → C, bottom). External amino acid residues are dark on white, and internal ones are white on dark (single-letter abbreviations).

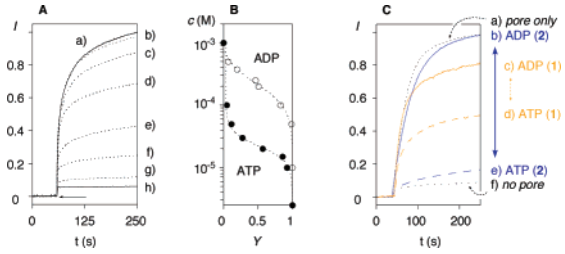

Figure 2. Discrimination of ATP and ADP by pores **1** and **2**. (A) Fractional change in CF emission I (λ_{ex} 492 nm, λ_{em} 517 nm) as a function of time after addition of ATP (0 (a), 1 (b), 10 (c), 20 (d), 30 (e), 50 (f), 100 (g), and 1000 μM (h) and barrel **2** (94 nM tetramer, arrow) to EYPC-LUVs⊃CF (65 μM EYPC, 10 mM HEPES, 107 mM NaCl, pH 6.5). (B) Dose-response curves for blockage of pore **2** by ATP (●) and ADP (○) with fit to the Hill equation. (C) As in A with 10 μM $MgCl_2$,[10] without (a) or with 100 μM ADP (b) and 100 μM ATP (e) for pore **2** compared to previous data[2] for blockage of pore **1** with equimolar amounts of ADP (c) and ATP (d).

In clear contrast to earlier results with synthetic multifunctional pore **1** (Figure 2Cc vs 2Cd), these studies demonstrated that it is possible to achieve the robust on−off ADP/ATP discrimination required for naked-eye sensing with synthetic multifunctional pore **2** (Figure 2Cb vs 2Ce). With this breakthrough in hand, it was straightforward to detect kinase activity.[2] In brief, D-glucose and ATP were reacted in the presence of hexokinase and, in strict analogy to chromatographic methods, aliquots were taken to test their ability to block pore **2** as a function of reaction time (Figure

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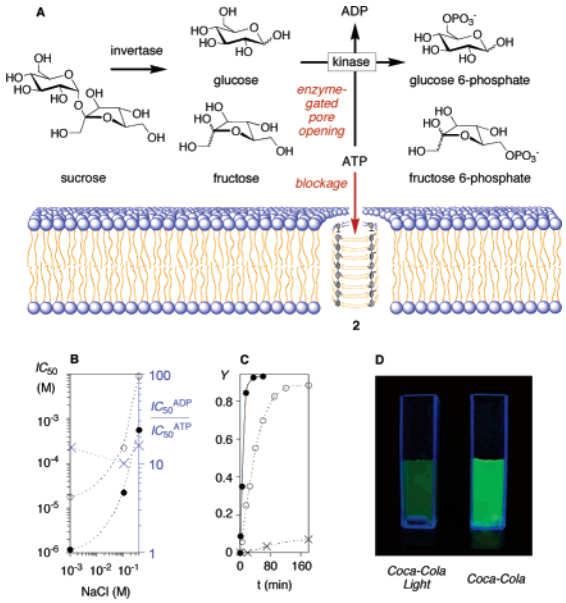

Figure 3. Dependence of ATP/ADP discrimination on ionic strength (B) and application of synthetic multifunctional pore **2** as detector of kinase activity (A, C) and as sugar sensor (A, D). (B) Dependence of IC_{50}^{ATP} (●), IC_{50}^{ADP} (○), and $IC_{50}^{ADP}/IC_{50}^{ATP}$ (×) on ionic strength, values determined as in Figure 2A.[8] (C) Fractional activity *Y* of pore **2** (94 nM) as a function of reaction time *t* of glucose (10 mM) and ATP (10 mM) in the presence of 0 (×), 0.24 (○) and 0.71 units/mL (●) hexokinase (10 mM $MgCl$,[10] 100 mM Tris, pH 8; 100× diluted with 10 mM HEPES, 107 mM NaCl, pH 6.5 for detection as in Figure 2). (D) Emission after addition of pore **2** (94 nM) to 1 mL of Coca-Cola Light (left) and Coca-Cola (right) diluted with (a) invertase (3×; 16 units/mL; 50 mM NaOAc/AcOH, pH 4.5, 55 °C, 10 min), (b) ATP (20×; 10 mM; 0.7 units/mL hexokinase, 10 mM $MgCl_2$, 100 mM Tris, pH 8, 30 °C, 40 min), and (c) EYPC-LUVs⊃CF (100×), excitation with UV lamp at 366 nm, detected ≤30 min after pore addition.

3A). The found pore opening during reaction was as expected for conversion of ATP to ADP during glucose phosphorylation (Figure 3C). The dependence of the velocity of pore opening on kinase concentration was in agreement with this interpretation.

Considering kinase as co-sensor, this finding identified synthetic multifunctional pore **2** as sensor of either glucose or ATP in mixed analytes, with the sensing selectivity being determined by the selectivity of the enzyme co-sensor. This concept of synthetic multifunctional pores as "universal sensors" was verified with sucrose sensing in soft drinks. For instance, treatment of Coca-Cola first with invertase to convert sucrose into glucose and fructose followed by phosphorylation of both hexoses[10] using hexokinase and ATP (Figure 3A) produced an ADP-rich mixture that did not interfere with the efflux of CF through pore **2** (Figure 3D, right). The complementary ATP-rich mixture was obtained from identical treatment of Coca-Cola Light (Figure 3D, left). Differentiation of Coca-Cola and Coca-Cola Light with the "naked eye" was as unproblematic as expected from the experimentally robust on−off ATP/ADP discrimination found for synthetic multifunctional pore **2** (Figure 3D). For quantitative analysis, dilution series were used to zoom in on the window available for the detection of pore opening in response to ATP consumption without interference from ADP (Figure 2B). All sucrose concentrations above these low micromolar ATP concentrations (plus the in part unavoidable dilution during sample preparation) were assessable with the pore sensor. Sucrose concentrations were then obtained from comparison with ATP calibration curves as in Figure 2B that included parameters such as time dependence and background correction for the unproblematic nonspecific CF efflux with fully blocked pores (Figure 2Ce vs 3D). Results for Coca-Cola and other beverages (Table 1) confirmed sugar sensing as a valid example for the practical applicability of basic research in the field of synthetic ion channels and pores.[11]

Table 1. Sucrose Content of Soft Drinks Determined with Pore **2**[a]

	beverage	expected (gL^{-1})	found (gL^{-1})
1	Coca-Cola	106	111 ± 7
2	Coca-Cola Light	0	0
3	Red Bull	113	118 ± 13
4	Fanta Orange	101	98 ± 9
5	Nestea Lemon	76	78 ± 7

[a] See Figure 1 for structures and Figure 3 for method.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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