pathway A of Figure 2 dominates over pathway B for the wild-type enzyme. Both H257G and F200G mutant PNPs catalyze N-9 to N-3 isomerization of inosine at a rate that is detectably competitive with the rate of phosphorolysis. For example $(k_{cat})_{phos}/k_{iso}$ = 240 was determined for the reaction catalyzed by F200G mutant human PNP. The N-9 to N-3 isomerization reaction is only observed when phosphate anion is bound to the oxocarbenium ion intermediate of these lid mutants. Therefore, the presence of bound phosphate anion causes an increase in the selectivity of the oxocarbenium ion intermediate for trapping by N-3 of hypoxanthine. The explanation for this effect of phosphate anion is unclear.

In summary, the innovative and clever approach adopted in this work of punching holes into human PNP shows that this dramatic change in enzyme structure has subtle and fascinating effects on the catalytic reaction mechanism. The results provide a snapshot of the enzyme-bound intermediate that falls short of crystal clarity, and they serve to emphasize the magnitude of the challenge of determining absolute rate constants for the reaction of unstable carbocation and carbanion intermediates within the restricted confines of an enzyme active site.

REFERENCES

Amyes, T.L., and Jencks, W.P. (1989). J. Am. Chem. Soc. *111*, 7888–7900.

Carpenter, B.K. (2004). In Reactive Intermediate Chemistry, R.A. Moss, M.S. Platz, and M.J. Jr, eds. (Hoboken, NJ: John Wiley & Sons), pp. 925–960.

Ghanem, M., Murkin, A.S., and Schramm, V.L. (2009). Chem. Biol. *16*, this issue, 971–979.

Lewandowicz, A., and Schramm, V.L. (2004). Biochemistry *43*, 1458–1468.

Richard, J.P. (1998). Biochemistry *37*, 4305–4309.

Saen-oon, S., Quaytman-Machleder, S., Schramm, V.L., and Schwartz, S.D. (2008). Proc. Natl. Acad. Sci. USA *105*, 16543–16548.

Wolfenden, R., and Snider, M.J. (2001). Acc. Chem. Res. *34*, 938–945.

Zechel, D.L., and Withers, S.G. (1999). In Comprehensive Natural Products Chemistry, C.D. Poulter, ed. (New York: Elsevier), pp. 279–314.

Reassembling Biological Machinery In Vitro

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Inspired by the specialized glycolytic system of flagella of mammalian sperm, Mukai et al. (2009) describe the controlled immobilization of two enzymes constituting the first steps in the glycolytic pathway. Extension of this work may provide "power converters" for bionanodevices, which transduce chemical energy from glucose to ATP.

Biomolecules are engineering marvels not only individually, but also in their collective operation within larger structures with complex functions. Research in nanobiotechnology aims to capitalize on biological structures directly by creating hybrid devices that merge biomolecules with artificial building blocks, and, indirectly, by investigating the engineering challenges faced by evolution as it improves molecular biosystems. The potential rewards of this approach include nanodevices with functionalities currently unattainable by manmade building blocks, and synthetic biology informed by engineering understanding.

An example of a biomolecular system redesigned and reassembled for engineering purposes is the motor protein-powered molecular shuttle (Hess et al., 2004). One design for such a nanoscale transport system with applications (e.g., in biosensing (Fischer et al., 2009)) relies on antibody-functionalized microtubules gliding on surface-adhered kinesin motors to capture and transport specific analyte molecules. These molecular shuttles are fueled by ATP, and the controlled and efficient provision of this fuel is a matter of ongoing interest.

Biological systems have evolved many mechanisms to secure the timely delivery of ATP at different locations throughout the cell. The glycolytic cycle couples ATP creation to a high energy fuel source—glucose, for example. To an engineer, the process resembles the conversion of AC high voltage at the wall outlet into DC low voltage used by electronic devices by one of the ubiquitous power supplies.

In this issue, Mukai and colleagues (2009) describe how they have been inspired by the controlled arrangement of glycolytic enzymes on the flagellar principal piece of mammalian sperm to pursue the engineering of a glycolytic ATP supply on a synthetic surface. The starting point of their investigation is the insight that the glycolytic enzymes in the flagellum are attached to the fibrous sheath via specific anchoring domains, and that these domains can be replaced by a His-tag via genetic engineering. These modified enzymes will then bind in specific orientations to synthetic surfaces rendered resistant to nonspecific protein adsorption but presenting Ni-NTA functional groups. By immobilizing at first two and then later all the enzymes of the glycolytic pathway, the ATP fuel can be

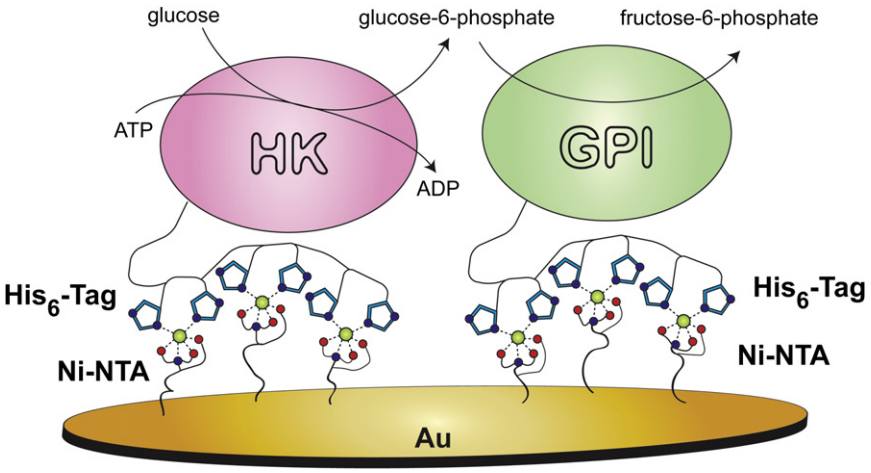

Figure 1. Controlled Immobilization of Two Enzymes of the Glycolytic Cycle
Two genetically engineered enzymes, hexokinase (HK) and glucose-6-phosphate isomerase (GPI), linked to a functionalized surface via His_6-tag/Ni-NTA interactions, perform the first two steps of the glycolytic pathway conversion of glucose to glucose-6-phosphate catalyzed by HK, followed by subsequent transformation of glucose-6-phosphate to fructose-6-phosphate, catalyzed by GPI.

generated from the glucose available in the environment at a specified location.

The present work (Mukai et al., 2009) describes the successful first steps in executing this strategy (Figure 1). Hexokinase and glucose-6-phosphate isomerase are genetically engineered to bind to Ni-NTA surfaces. While nonspecific binding is not completely suppressed, the enzymes binding through their His-tags in specific orientations are shown to have a several-fold higher activity. Furthermore, if both enzymes are bound to the same surface, the sequential conversion of glucose into glucose 6-P and fructose 6-P is demonstrated. These results demonstrate that multistep enzymatic pathways can be reconstituted on solid supports.

It will be interesting to further work out the implications of the placement of multiple enzymes with high activity on a solid surface for mass transport and stability. Furthermore, enzymes may be joined by immobilized cofactors (Liu et al., 2009), and the localization of the generated fuel (Tucker et al., 2008) may be aided by adding sequestration enzymes in specific patterns.

The reconstitution of the glycolytic cycle on a surface would be of significant benefit to a hybrid nanodevice because glucose, rather than ATP, is provided by the blood stream, making a "power converter" essential. The provision of a molecular shuttle with ATP derived from a single enzymatic reaction occurring on an enzyme-coated microsphere carried by the shuttle was an exciting first step in this direction (Du et al., 2005). Another suggestion is to integrate isolated mitochondria as power converters into such systems (Wasylycia et al., 2008). However, the idea of Mukai et al. (2009) to immobilize an entire pathway on a surface combines generality with tunability, and is an advance in the battle to support protein function within artificial environments (Fischer and Hess, 2007).

REFERENCES

Du, Y.Z., Hiratsuka, Y., Taira, S., Eguchi, M., Uyeda, T.Q.P., Yumoto, N., and Kodaka, M. (2005). Chem. Commun. (Camb.) *16*, 2080–2082.

Fischer, T., Agarwal, A., and Hess, H. (2009). Nat. Nanotechnol. *4*, 162–166.

Fischer, T., and Hess, H. (2007). J. Mater. Chem. *17*, 943–951.

Hess, H., Bachand, G.D., and Vogel, V. (2004). Chemistry *10*, 2110–2116.

Liu, W., Zhang, S., and Wang, P. (2009). J. Biotechnol. *139*, 102–107.

Mukai, C., Bergkvist, M., Nelson, J.L., and Travis, A.J. (2009). Chem. Biol. *16*, this issue, 1013–1020.

Tucker, R., Katira, P., and Hess, H. (2008). Nano Lett. *8*, 221–226.

Wasylycia, J.R., Sapelnikova, S., Jeong, H., Dragoljic, J., Marcus, S.L., and Harrison, D.J. (2008). Lab Chip *8*, 979–982.

