M2 Masters in systems and synthetic biology
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Reactome array: forging a link between metabolome and genome

*Science*. 2009 Oct 9;326(5950):252-7; Retraction: *Science* 2010 Nov 12;330(6006):912

This paper has been the subject of both excitement and condemnation. Even after its retraction, there are top scientists who maintain the reactome array functions as described ann there is a company that sells the technology (Enzymolome array): http://reactomix.com. Here we revisit this study 5 years later to examine what opportunities exist for this technology.

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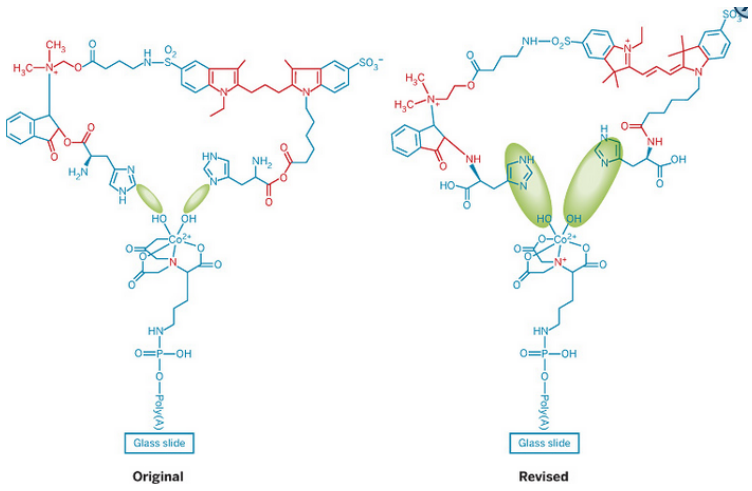
1. Fig 1 below shows the chemical complex attached to the array surface in both the originally published (left) and corrected (right) versions.

1A. Describe the chemical transformations that occur when an enzyme reacts with its substrate on the reactome array (2 pts).

An enzyme-catalyzed chemical change in the substrate at a position adjacent to the weakly amine region causes rupture of the labile nitrogen:metabolite bond and release of the quenched Cy3 dye. This in turn provokes release of the reaction product and the histidine “tags” anchored to the Co(II), thereby exposing an active cobalt cation that ligates and immobilizes the enzyme on the array spot. The released dye is no longer quenched and gives a fluorescent signal.

1B. Much of the initial controversy focused on errors in the chemical structure in Fig 1. What were their mistakes and how were they corrected? Please be as specific as possible with respect to molecules and bonds (2 pts).

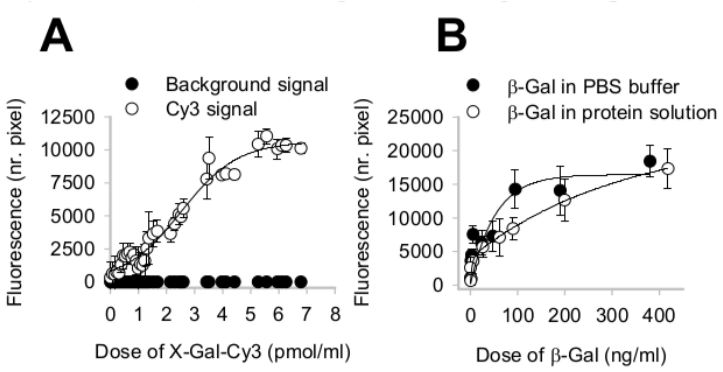



http://cen.acs.org

Histidine is linked to the substrate and the Cy3 linker by its C-terminus in the original and by its N-terminus in the corrected version. The Cy3 amino linker is missing a carbon in the original version. The original version is missing carbons on both Cy3 indol groups, a positive charge on a nitrogen atom, and double bonds connecting the indols.

2. The molecular complex on the array surface has 3 linked components, one of which is a quenched Cy3. Briefly, what is “fluorescence quenching”? Your answer does not need to include a chemical mechanism (2 pts).

Quenching refers to any process which decreases the fluorescence intensity of a given substance. A variety of processes can result in quenching, such as excited state reactions, energy transfer, complex-formation and collisional quenching.




3. Fig S2: Describe how these two figures correlating Cy3 fluorescence with X-gal-Cy3 **A** and B-gal **B** concentrations support the function of the reactome array (2 pts).

Panel A shows Cy3 fluorescence is positively correlated with X-gal-Cy3 concentration at constant B-gal concentration. Panel B shows Cy3 fluorescence is positively correlated with B-gal concentration at constant X-gal-Cy3 concentration. Together, these results support that the reaction between B-gal (enzyme) and X-gal (substrate) liberates Cy3 to enable its fluorescence, as occurs on the reactome array.

> 3A. Why was it important to show increases in fluoresence for both B-gal in PBS (pure protein) and B-gal in an *E.coli* lysate (protein solution) in panel B above? (1 pt).

It is important that Cy3 fluorescence depends on B-gal concentration when mixed with a cell lysate in order to demonstrate that the reactome array can detect a specific enzyme activity even in the presence of other, competing enzymes.

4. In their experimental design, the array is first used to identify metabolites that act as substrates for enzymes present in a cell lysate. The enzymes that react with these metabolites are then identified using metabolite-coated gold nanoparticles. What are the steps required to identify proteins using these nanoparticles? (4 pts).

The nanoparticle (NP) coated with a single metabolite complex is allowed to react with the cell lysate. An enzyme able to transform the substrate becomes captured by the Co(II) cation of the linker and immobilized on the NP. After recovery of the NP by centrifugation, and washing to remove unbound enzymes, the specifically captured enzyme(s) is released by imidazole treatment of the NP, separated in pure form from the NP by filtration, sequenced, and functionally characterized.

5. The enzymes identified using gold nanoparticles are characterized by their Kcat/Km (Fig 2C). What do Kcat, Km, and Kcat/Km measure? (3 pts).

Kcat: Vmax/[enzyme] (1/sec) is the maximum number of chemical conversions of substrate molecules per second that a single catalytic site will execute for a given enzyme concentration.

Km: the substrate concentration at which the reaction rate is half of Vmax. Enzymes with a low Km have high affinity for the substrate.

Kcat/Km: catalytic efficiency of the enzyme, i.e how fast in M/seconds the enzyme reacts once it encounters the substrate. Usually, the upper limit of Kcat/Km is diffusion because the substrate has to diffuse and collide with the enzyme before it can be converted into product.

6. They apply the reactome array to study the metabolic potential of microbial samples from the environment. However, environmental samples often do not contain enough cells to react with the array. What is their 2 step solution to this problem? What is a potential problem with this solution? (2 pts).

To obtain sufficient genomic DNA for library construction, they first stimulated cell growth in the samples by adding growth substrates. They then harvested the cells, extracted their total DNA, and established metagenome libraries in *E. coli*. A potential problem with the first step is that growth in the lab might have changed the community composition such that the sample they studied differs from the natural community. Potential problems with the second step of cloning and expressing the DNA in *E.coli* relate to how not all genes clone and express in *E.coli.*

7. How does this figure (Fig S14) support that the method discussed in question 6 was justified? (2 pts).

Fig S14 shows that reactome fluorescence for individual enzyme activities is very similar for *P. putida* enzymes expressed in in the native host (x-axis) and for these enzymes cloned and expressed in *E. coli* (y-axis). This supports that cloning DNA from environmantal samples into *E.coli* does not significantly change the activity measurements on the reactome array.