

Background

The past decade has seen an increasing number of investigations into enhanced diffusion of catalytically active enzymes. These studies suggested that enzymes are actively propelled as they catalyze reactions or bind with ligands (e.g., substrates or inhibitors) and thus lead to a 30-80% increase in their diffusivity. These experiments have been conducted with different enzymes (catalase, urease, alkaline phosphatase, aldolase, etc.) using mostly fluorescence correlation spectroscopy (FCS), and also stimulated emission-depletion fluorescence correlation spectroscopy (STED-FCS) and single molecular tracking.

However, the origin of the enhanced diffusion still remains controversial, and the existing observations are not sufficient to accept or refute if any enzyme can work as an active motor due to catalysis.



Fig 1. Schematic of enhanced diffusion of enzymes during catalysis

Past observations have artifacts

We found that some diffusion coefficients obtained from FCS measurements are significantly different from the reported values and the classic prediction made by the Young-Carroad-Bell model. This could be attributed to the dissociation of multimeric enzymes, adsorption to surfaces, conformational changes, and fluorophore quenching.

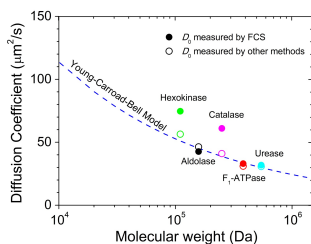


Fig 2. Comparison of diffusion coefficients of free enzymes measured by FCS and by other methods

Aldolase shows no enhanced diffusion in DLS

Aldolase, an endothermic enzyme, was reported to show 30% higher diffusivity in the presence of its substrate fructose-1,6-bisphosphate (FBP) in FCS measurements.

We use aldolase with the highest activity ($k_{cat} = 32 \text{ s}^{-1}$ and $K_m = 10 \text{ µM}$) compared with that used in similar studies and dynamic light scattering (DLS, with an experimental error of 3%) to determine the diffusion coefficient of aldolase.

We did not observe any enhanced diffusion by DLS. The slight decrease in the diffusion coefficient in 50 mM FBP is mainly due to the increased viscosity of the solutions.

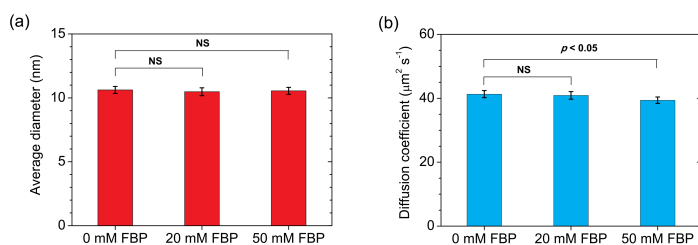


Fig 3. (a) Average hydrodynamic diameters and (b) diffusion coefficients of aldolase in the absence and presence of 20 and 50 mM FBP.

No clear correlation to enzyme turnover rates

One of the hypotheses is that the enhanced diffusion is correlated to the turnover events. However, diffusion enhancement does not show a clear dependence on the enzyme turnover number (Fig. 4). Recent experiments found no enhanced diffusion for aldolase, alkaline phosphatase and triose phosphate isomerase, conflicting with previous investigations.

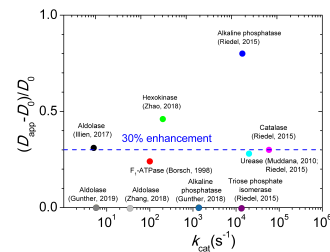


Fig 4. Reported diffusivity enhancements of active enzymes as a function of turnover numbers

Conformational changes are not Sufficient

We assessed how significant the gyration radius (R_g) of enzymes (or proteins) could change upon substrate binding. Most enzymes, even those experience large conformational changes, cannot decrease its hydrodynamic size by more than 20% (Fig. 5 and Table 1).

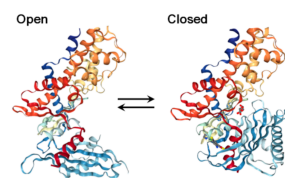


Fig 5. Large conformational changes of a clamp-shaped enzyme, human glucokinase, only decrease its R_g by 6%.

Table 1. R_g changes of some enzymes/proteins upon substrate binding

Enzyme	Substrate (or ligand)	$R_{g,free}/R_{g,bound}$	Enzyme	Substrate (or ligand)	$R_{g,free}/R_{g,bound}$
Lobster arginine kinase	arginine + Mg-ADP	1.06	Creain kinase from mitochondria	Mg-ATP	1.14
L-Arabinose-binding protein	L-arabinose	1.05	Creain kinase from muscle	creatine	1.01
Yeast hexokinase (II isozyme, monomeric)	glucose	1.04		Mg-ATP	1.09
	glucose 6-phosphate	1.05		creatine	0.99
Human glucokinase (hexokinase IV)	glucose + AMP-PNP	1.06		glucose 6-phosphate	1.05
Rabbit muscle pyruvate kinase	P-enolpyruvate	1.02	Yeast malate synthase	acetyl-CoA	1.04
	L-phenylalanine (inhibitor)	0.98		acetyl-CoA + pyruvate	1.05

Conclusion and outlook

1. Our findings challenge the idea that enzymes can be self-propelled by their catalytic activity.
2. The past observations of enhanced diffusion of enzymes need to be reexamined experimentally and theoretically.

Reference and acknowledgment

Reference

- [1] Y. Zhang, H. Hess. *ACS Central Science*, 5(6), 939-948 (2019).
- [2] Y. Zhang, M.J. Armstrong, N.M. Bassir Kazeruni, H. Hess. *Nano Letters* 18(12), 8025-8029 (2018).

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