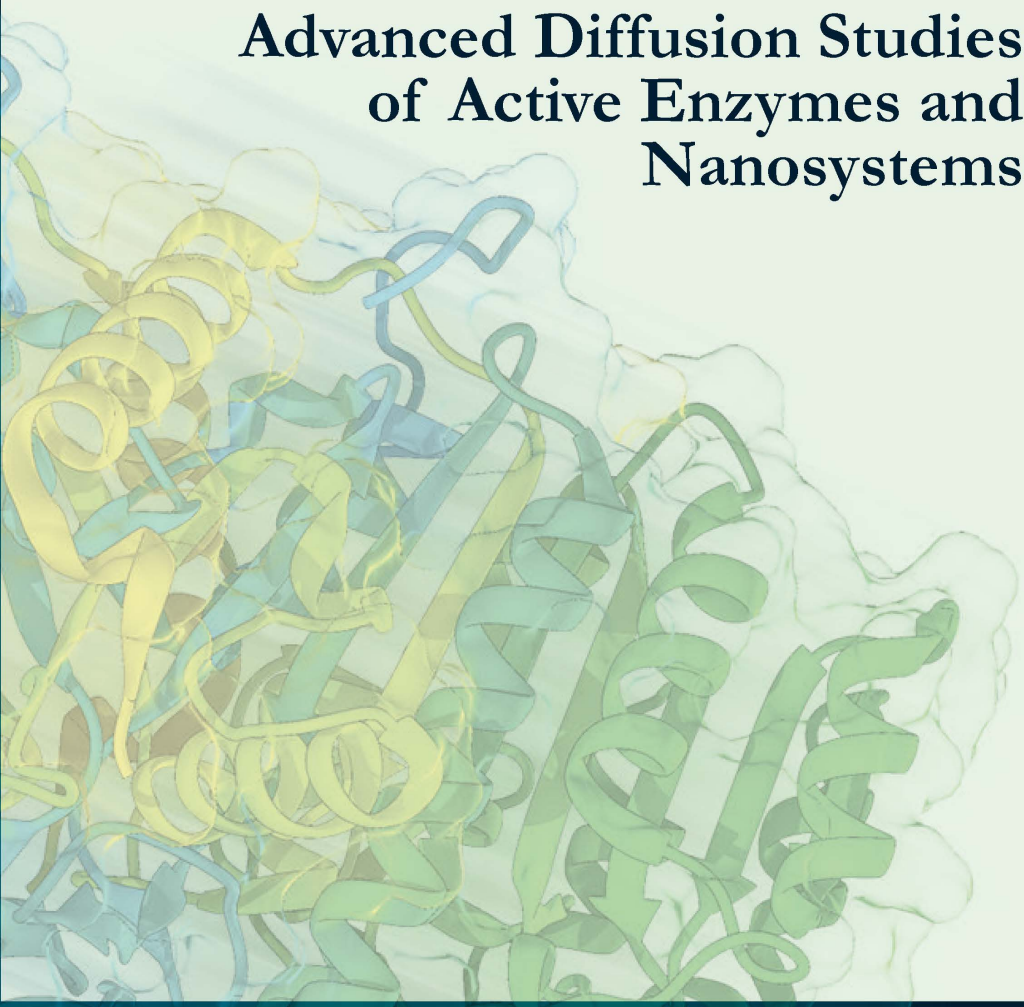
Jan-Philipp Günther

Advanced Diffusion Studies of Active Enzymes and Nanosystems



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Advanced Diffusion Studies of Active Enzymes and Nanosystems

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Jan-Philipp Günther

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Max-Planck-Institut für Intelligente Systeme, Stuttgart

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To my family

"Love is just a chemical. We give it meaning by choice."[6]

Eleanor Lamb

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Abbreviations

3HT	His-tag modified bacteriophages (pIII coat protein)
ABEL	anti-Brownian electrophoretic
ACF	autocorrelation function
ALD	fructose-bisphosphate aldolase
AMPPNP	β,γ-imidoadenosine-5'-triphosphate
APD	avalanche photo diode
ATP	adenosine triphosphate
b	diffusion weighting factor
$\boldsymbol{B}$	magnetic field
B_0	static magnetic field strength
B_1	oscillating magnetic field strength
D	(translational) diffusion coefficient
D_0	diffusion coefficient in absence of interactions
D_{app}	apparent diffusion coefficient
D_{eff}	effective diffusion coefficient
D_{rot}	rotational diffusion coefficient
δ	chemical shift
δ_G	gradient pulse length
Δ	diffusion time
ΔE	energy difference
$\Delta \boldsymbol{x}^2$	mean-square-displacement
DHAP	dihydroxyacetone phosphate
DLS	dynamic light scattering
DNA	deoxyribonucleic acid
E-P-C	enzyme-phage-colloid
η	viscosity
$\boldsymbol{F}$	force
FBP	fructose-1,6-bisphosphate
FCCS	fluorescence cross correlation spectroscopy
FCS	fluorescence correlation spectroscopy
FID	free induction decay
FPLC	fast protein liquid chromatography

Abbreviations

FRET	Förster resonance energy transfer
G	autocorrelation function (FCS)
G	gradient-pulse strength (NMR)
G3P	glyceraldehyde 3-phosphate
GDH	α-glycerophosphate dehydrogenase
γ	gyromagnetic ratio
h	Planck constant
$\boldsymbol{I}$	angular momentum
I	spin quantum number (NMR theory)
I	fluorescence/echo signal intensity (FCS/NMR)
I_0	signal intensity in absence of quencher/gradient (FCS/NMR)
ISC	intersystem crossing
k	process rate
$\mathrm{k_B}$	Boltzmann constant
k_{cat}	catalytic reaction rate
L	length of a swimmer
λ	boundary layer thickness (phoretic theory)
λ	wavelength (optical spectroscopy)
Λ	combined interaction parameter
$\boldsymbol{M}$	macroscopic magnetization
$\boldsymbol{M}_0$	equilibrium magnetization
m_I	magnetic quantum number
μ	individual magnetic moment
μ	Stokes drag coefficient
N	average number of fluorophores
∇	nabla operator
NADH	nicotinamide adenine dinucleotide
NHS	N-hydroxysuccinimide
NMR	nuclear magnetic resonance
NPP	p-nitrophenylphosphate
ν_0	Larmor frequency
ω_0	radius in radial direction
p	pressure
P	product
PAGE	polyacrylamide gel electrophoresis

PBS	phosphate-buffered saline
PDB	protein data bank
PFG-NMR	pulsed field gradient-NMR
PP	pyrophosphate
ψ	interaction potential
Q	quencher molecule
R	radius
R_h	hydrodynamic radius
Re	Reynolds number
RF	radio frequency
ρ	density
S	substrate (reactant)
S	singlet state (Jablonski diagram)
SDS	sodium dodecyl sulfate
SPT	single particle tracking
ss	single-stranded
STED	stimulated emission depletion
sulfo-SIAB	sulfosuccinimidyl(4-iodoacetyl)aminobenzoate
T	absolute temperature
T	fraction of molecules in the triplet state (FCS)
t	time
T_1	spin-lattice relaxation constant
T_2	spin-spin relaxation constant
τ	lag time
τ_D	translational diffusion time
τ_F	fluorescence lifetime
τ_R	correlation time (NMR relaxation)
τ_T	triplet relaxation time
TCSPC	time-correlated single photon counting
TPI	triosephosphate isomerase
Tris-d_{11}	tris(hydroxymethyl-d_3)amino-d_2-methane
$\boldsymbol{u}$	mass flow velocity
v	swimming speed
z_0	radius in axial direction

Abstract

Enzymes are fascinating nanomachines, which catalyze the reactions essential for life. Studying enzymes is therefore important in a biological and medical context, but the catalytic potential of enzymes also finds use in organic synthesis. This thesis is concerned with the fundamental question whether the catalytic reaction of an enzyme can cause it to show enhanced diffusion. Additionally, this thesis examines if it is possible for enzymes to collectively affect fluid flow, when they are incorporated in functional biohybrid nanostructures.

The diffusive behavior of enzymes is important for their distribution within cells and impacts their ability to reach their substrates. Several reports within the last decade have claimed that enzymes show diffusion enhancements of up to 80 %, when they are catalytically active. Examining these claims is important to achieve an understanding of their biological function. These reports were based on fluorescence correlation spectroscopy (FCS), which measures the fluorescence fluctuations of individual fluorophores (here, labeled enzymes) passing through a small focal volume. FCS is a powerful tool to study diffusion, but several photo- and biophysical processes can interfere with FCS measurements. The work presented in this thesis made theoretical predictions how these effects can lead to misinterpretations specific to FCS experiments of active enzymes. Additionally, these simulations were supported by multi-detector FCS experiments, which showed that the 80 % diffusion enhancement reported by others is actually a misinterpretation of a complex fluorescence quenching artefact. The FCS experiments reported within this thesis find no evidence for active enzyme diffusion enhancement.

To completely rule out the possibility of fluorescence artefacts, another diffusion measurement technique, which does not require labeling, was adapted for enzymes: Diffusion nuclear magnetic resonance (NMR) spectroscopy. Hence, the work presented in this thesis reports the first diffusion NMR experiment of an active enzyme. Earlier reports by others claim that enzymes, which catalyze endothermic reactions, self-propel and cause enhanced diffusion of molecules in their surroundings. The diffusion NMR studies in this

thesis reveal that neither the enzymes themselves nor tracer molecules in the solution show enhanced diffusion.

These findings of enzyme diffusion NMR measurements and the unraveling of artefacts in FCS enzyme measurements seriously question the hypothesis that enzymes are active matter and experience enhanced diffusion. The publication of these results was the first to experimentally question this hypothesis. Since then, several reports by others have appeared that support the predictions and experimental observations presented herein.

In addition to the academic interest, there is also a practical interest in enzymes due to their fascinating properties as catalysts. Biocatalysis, which uses enzymes or whole organisms for organic synthesis, has several advantages over conventional catalysis. Enzymes are, for instance, highly selective and efficient, can be operated in mild conditions and are safe to dispose of. The natural enantioselectivity of enzymes in some reactions is of special interest for the synthesis of pharmaceuticals, which are often homochiral. However, reuse of enzymes by recovery from reaction mixtures is problematic due to their small size and fragility. Immobilization of enzymes onto microparticles simplifies the recovery step, but often lowers the enzymes' catalytic activity. In this thesis, a novel nanoconstruct is presented, which allows easy recovery of enzymes with a magnet, but still ensures high enzymatic activity. In this construct, filamentous viruses are utilized as intermediate immobilization templates between the microparticle and the enzyme. This novel nanoconstruct has been termed enzyme-phage-colloid (E-P-C). Within this thesis two applications for E-P-Cs are presented. The first application is the repeated use of E-P-Cs as biocatalysts with easy magnetic recovery between each reaction cycle. Activity assays showed that the enzyme had even higher catalytic turnover, when it was immobilized on the E-P-C. In the second application, E-P-Cs were used to construct an enzymatic micropump, which is a microdevice that creates convective flows due to density differences with a locally catalyzed reaction. E-P-Cs can conveniently be immobilized with a magnet onto the wall of a microcontainer to form an enzymatic micropump. These E-P-C micropumps are shown to generate the fastest flow speeds of an enzymatic micropump to date. Additionally, it is shown that urease E-P-Cs can pump blood at physiological urea concentrations, which might enable medical lab-on-a-chip applications.

The last part of this thesis considers the diffusion of active molecular catalysts. These synthetic catalysts have recently been reported to show enhanced diffusion in diffusion NMR experiments similar to enzymes. This is particularly surprising as the molecular catalysts exhibit catalytic turnover rates that are orders of magnitude lower than the ones of enzymes. In addition to the proposed self-propulsion, it was claimed that the molecular catalysts transfer kinetic energy to the surrounding solvent and substrate molecules, thereby causing their diffusion enhancement. However, re-examination of these diffusion NMR studies showed that there is no diffusion enhancement of molecular catalysts and the misinterpretation of the diffusion NMR experiments is caused by a complex artefact due to intensity changes over the course of the diffusion experiment, which are caused by relaxation phenomena. These results have recently been submitted for publication.

Kurzzusammenfassung

Enzyme sind faszinierende Nanomaschinen und katalysieren alle Reaktionen, die essentiell für das Leben sind. Die Forschung an Enzymen ist daher notwendig für die Biologie und Medizin, aber die katalytischen Fähigkeiten von Enzymen finden auch Anwendung in der organischen Synthese. Diese Arbeit befasst sich mit der Frage, ob die enzymatische Katalyse bei Enzymen selbst erhöhte Diffusion hervorrufen kann. Außerdem untersucht sie, ob es möglich ist für Enzyme kollektiv Strömungen in Flüssigkeiten hervorzurufen, wenn sie in funktionale Biohybrid-Nanostrukturen eingebunden sind.

Das Diffusionsverhalten von Enzymen ist wichtig für ihre Verteilung in Zellen und beeinflusst die Fähigkeit von Enzymen Substrate zu erreichen. Mehrere Berichte innerhalb des letzten Jahrzehnts behaupten, dass Enzyme ihre Diffusion um bis zu 80 % erhöhen, wenn sie katalytisch aktiv sind. Dies hätte große Auswirkungen auf unser Verständnis der biologischen Funktion von Enzymen. Die erwähnten Berichte basieren auf Messungen der Fluoreszenzkorrelationsspektroskopie (FCS), bei der die Fluoreszenzfluktuationen einzelner Fluorophore (hier, fluoreszenzmarkierte Enzyme) gemessen werden, während diese sich durch ein kleines fokales Volumen bewegen. FCS ist eine wertvolle Methode für Diffusionsmessungen, wird aber von foto- oder biophysikalischen Prozessen beeinflusst. Diese Arbeit beinhaltet theoretische Voraussagen darüber wie diese Prozesse zu Fehlinterpretationen speziell im Fall von FCS Messungen aktiver Enzyme führen können. Zusätzlich wurden diese Simulationen von Multidetektor-FCS Messungen bestätigt, was zu der Erkenntnis führte, dass die berichteten 80 % Diffusionserhöhung tatsächlich auf einer Fehlinterpretation eines komplexen Fluoreszenzlöschungsartefakts basieren. Die FCS Messungen in dieser Arbeit zeigen daher keine Diffusionserhöhung aktiver Enzyme.

Um Fluoreszenzartefakte komplett ausschließen zu können, wurde eine weitere Methode zur Diffusionsmessung, welche keine Probenmarkierung benötigt, auf Enzymmessungen erweitert. Hierbei handelt es sich um die Methode der Diffusionskernspinresonanz-spektroskopie (Diffusions-NMR-Spektroskopie). Diese Arbeit beinhaltet daher die ersten Diffusions-NMR-Messungen aktiver Enzyme. Frühere Arbeiten anderer Forschungsgruppen