

Smart Polymers

Photoresponsive polymer-enzyme switches

Smart Polymers

1. Einführung in die Technik

- Was sind smart Polymers?
 - Warum der Begriff smart oder intelligent?

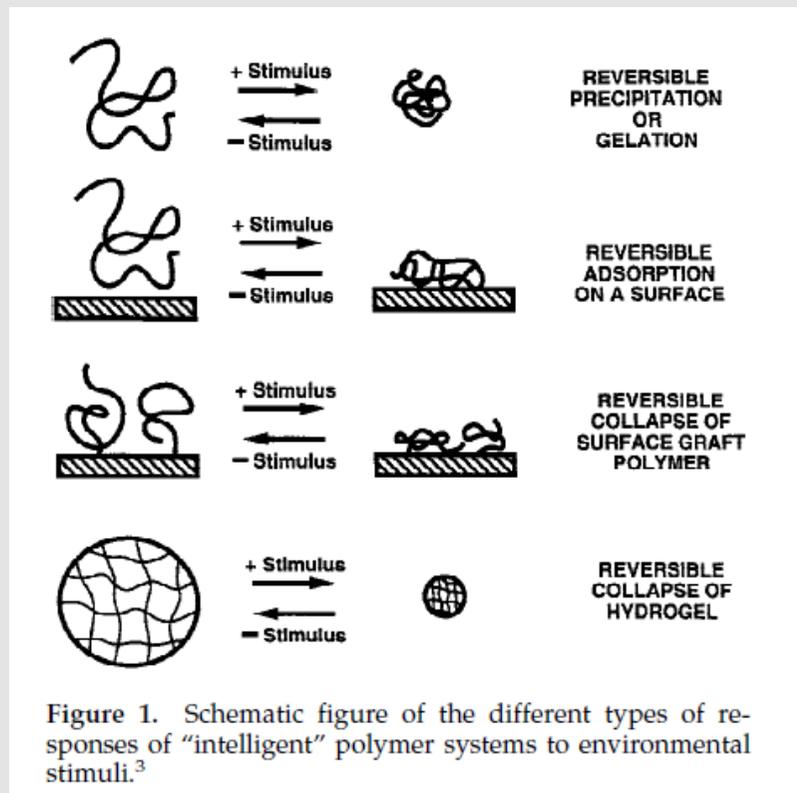
Table I
Environmental Stimuli

Physical	Chemical	Biochemical
Temperature	pH	Enzyme substrates
Ionic strength	Specific ions	Affinity ligands
Solvents	Chemical agents	Other biochemical agents
E.M. radiation (UV, visible)		
Electric fields		
Mechanical stress, strain		
Sonic radiation		
Magnetic fields		

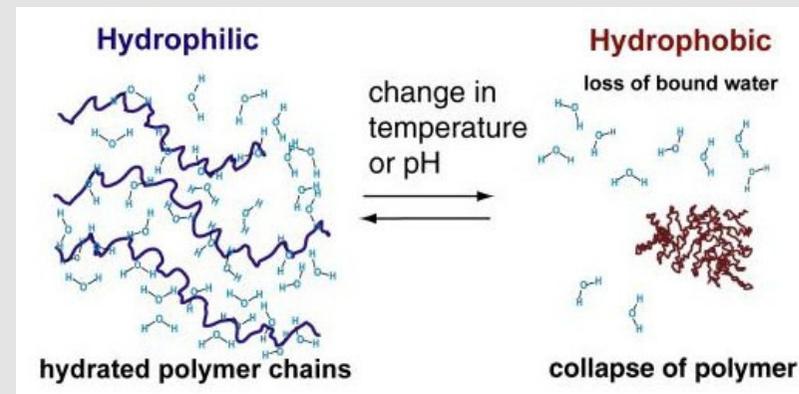
Reaktion auf äußere Reize

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1. Einführung in die Technik



Phasenseparation als Reaktion auf eine Änderung der umgebenden Bedingungen



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1. Einführung in die Technik

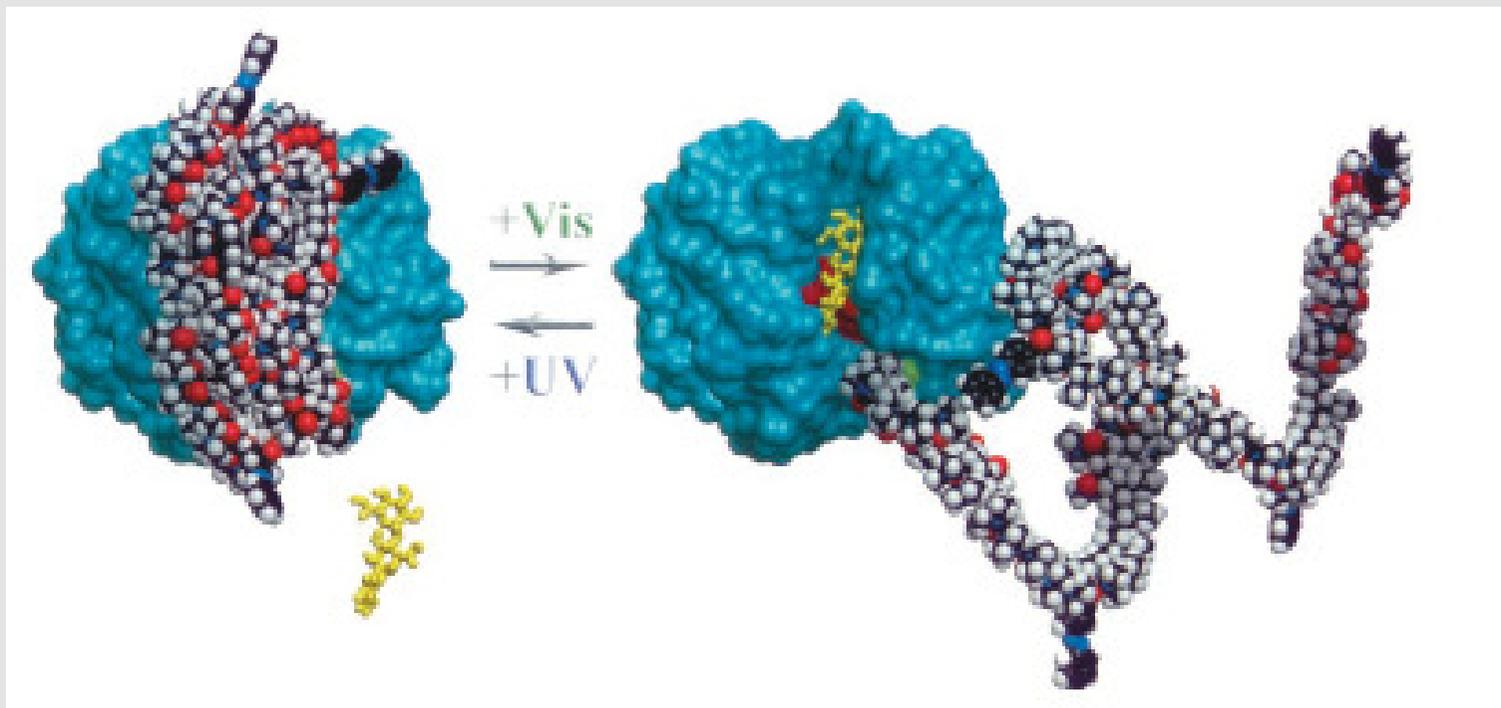
Warum ist diese Methode nützlich?

- Anwendungen in der Medizin und Biotechnologie
 - drug delivery. Smart polymers matrices
 - Hydrogele, Bioseparation
 - Immuntests
 - rekombinante Proteine, zB Steuerung von Ligand oder Zellbindung

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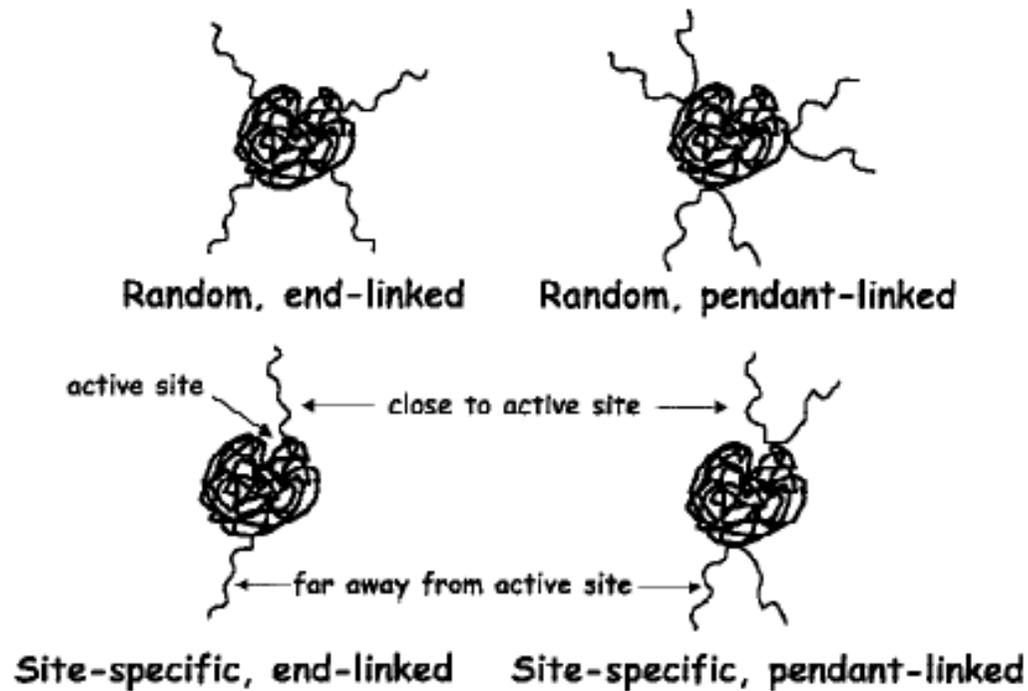
1. Einführung in die Technik

Biokonjugate



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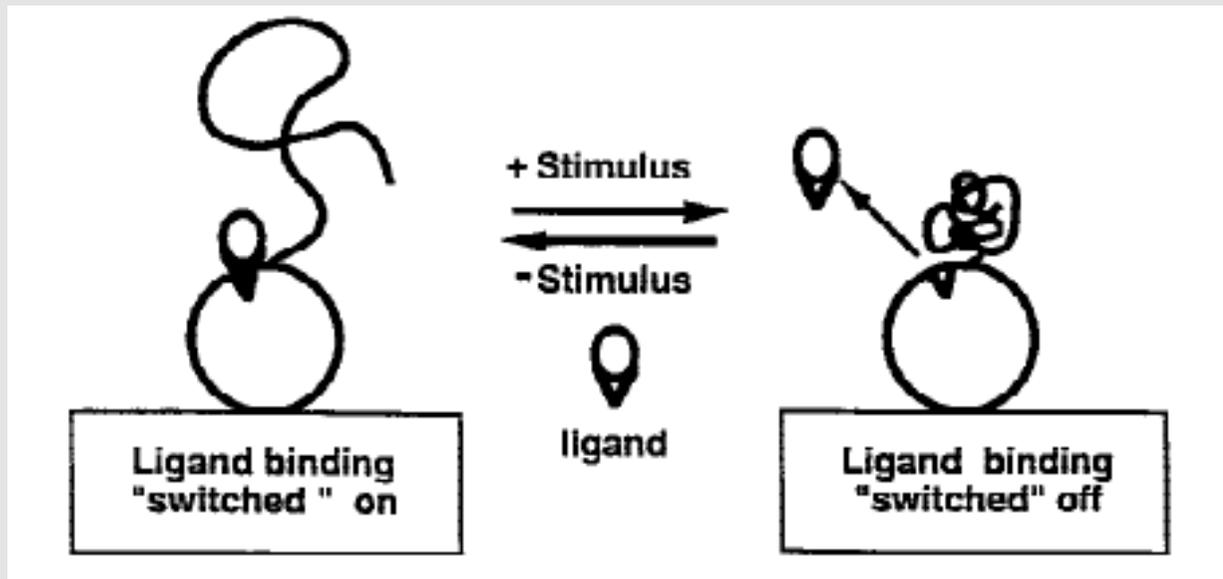
2. Photoresponsive polymer-enzyme switches



mögliche Bindungen

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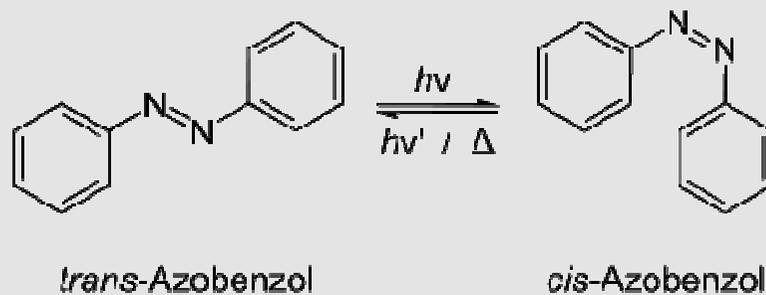
2. Photoresponsive polymer-enzyme switches



Photoswitches

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2. Photoresponsive polymer-enzyme switches



Photoswitches:

- absorbiertes Licht aktiviert eine chem. Transformation, zB. Photoisomeration
- dient hier als molekulare Antenne, bzw. molekularer Schalter

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2. Photoresponsive polymer-enzyme switches

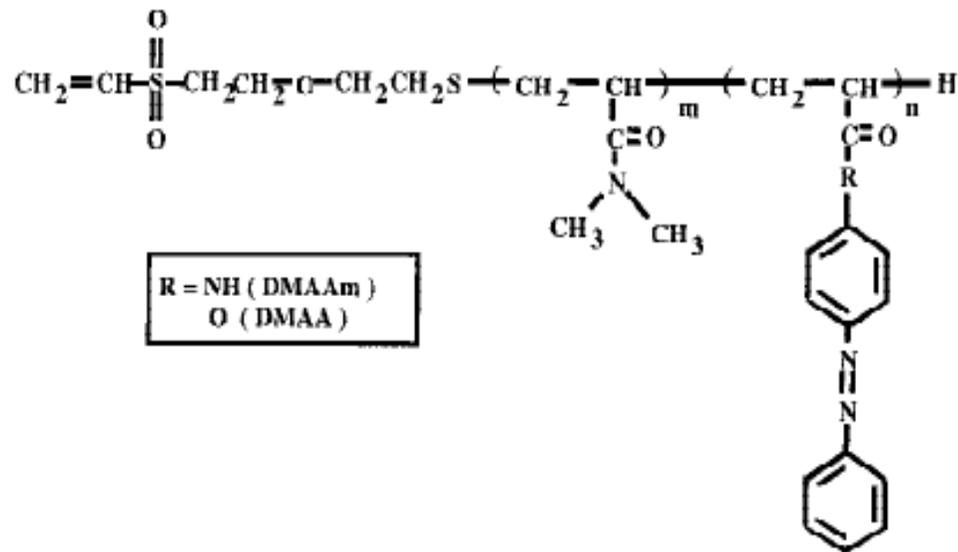


Figure 11. Composition of the light-sensitive copolymers that have been conjugated to strepavidin. ⁴¹

Synthese der licht empfindlichen Polymere

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2. Photoresponsive polymer-enzyme switches

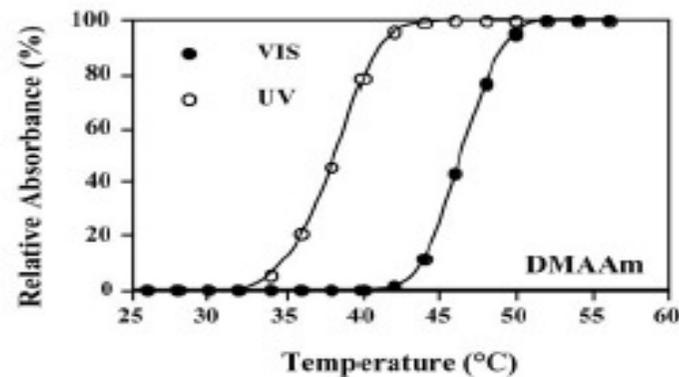
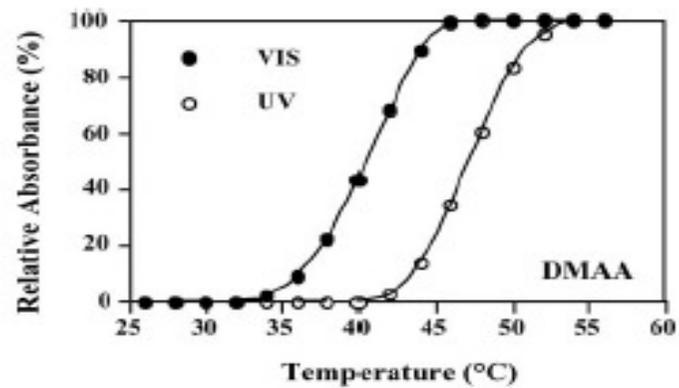


Fig. 2. The LCST behavior under UV and VIS photoirradiation of the DMAA and DMAAm polymers used to construct EG 12A conjugates. The polymer concentration was 2 mg/ml in 100 mM phosphate buffer, pH 7.2.

LCST Verhalten unter UV bzw Vis Bestrahlung

Messtemperatur 45°C!

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2. Photoresponsive polymer-enzyme switches

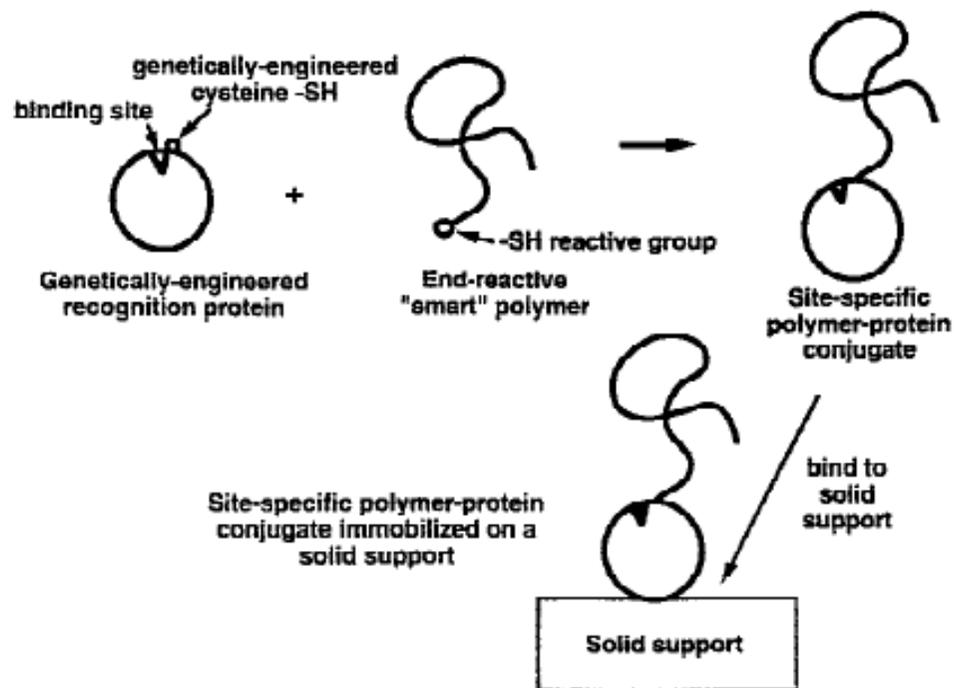


Figure 5. Schematic of the process for preparing a site-specific conjugate of a smart polymer with a genetically engineered mutant protein.

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2. Photoresponsive polymer-enzyme switches

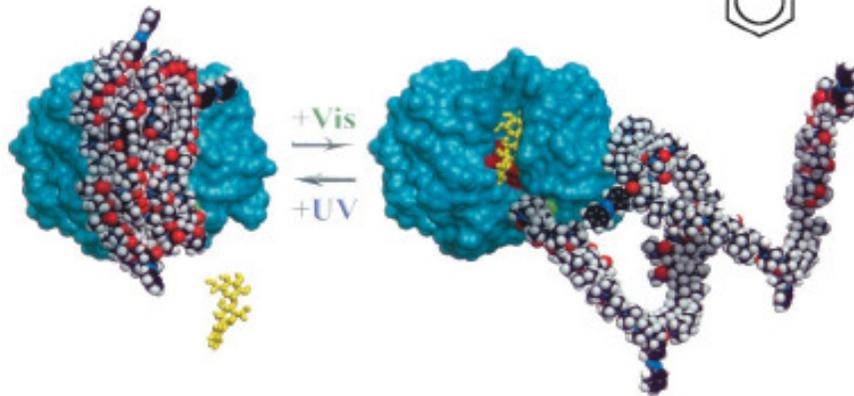
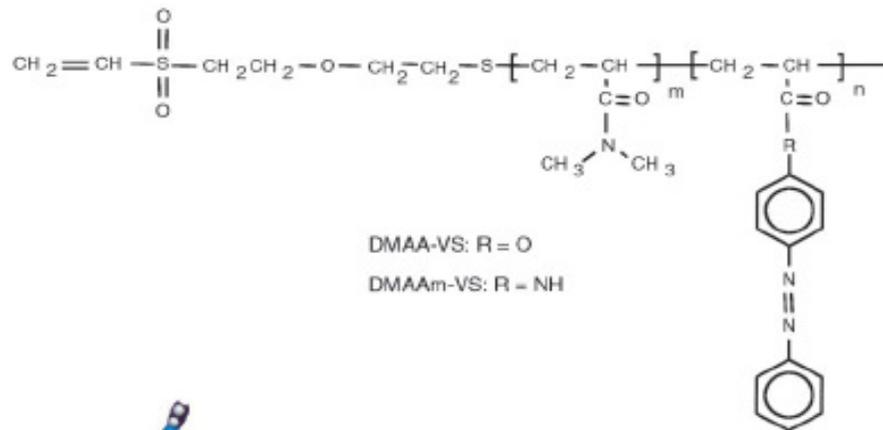


Fig. 1. Schematic model for the photoresponsive enzyme switch. The photoresponsive copolymer compositions are shown with the end-modified vinyl sulfone terminus for thiol-specific conjugations. The 3D model of EG 12A displays the relative locations of position 55 (colored green with schematic polymer coil attached) and the catalytic active site residues D99, E116, and E200 shown in red. The ONPC substrate is shown schematically to show the orientation of the active site groove. The polymer coil is shown as a 10-kDa chain with a distribution of nine DMAA monomers to one azobenzene monomer.

Endoglucanase
Mutant N55C
konjugiert mit DMAAm

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2. Photoresponsive polymer-enzyme switches

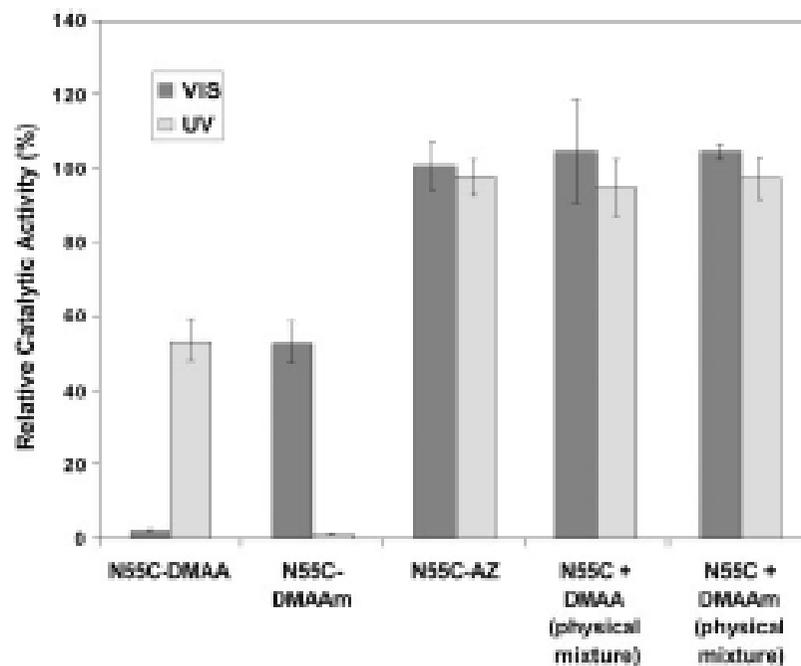


Fig. 3. Photoinduced activity changes of N55C-DMAA, N55C-DMAAm, and N55C-AZ conjugates, along with control physical mixtures of the polymers and N55C EG 12A. The activity was measured for 100 nM conjugates using 8 mM ONPC as a substrate in 50 mM sodium acetate buffer, pH 5.5, at 45°C, and normalized to the activity of the unconjugated N55C EG 12A.

Katalytische Aktivität des N55C

Messtemperatur 45 °C

Substrat: ONPC

Produkt: Nitrophenol

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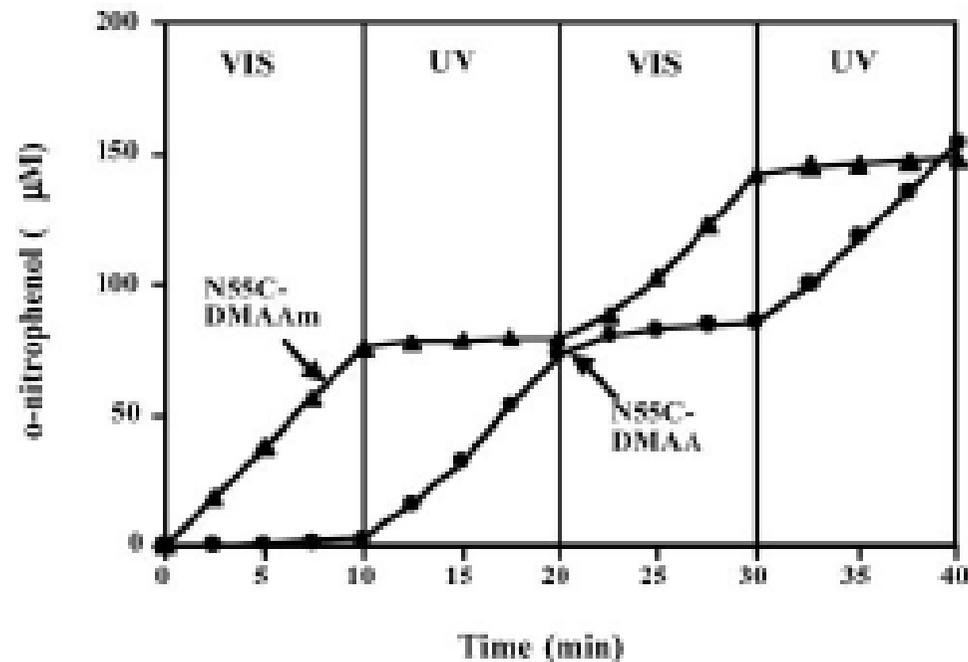


Fig. 4. Sequential photoswitching of the activity of the N55C-DMAA and N55C-DMAAm conjugates. The product (*o*-nitrophenol) concentration was measured for 100 nM conjugates with free polymer present, using ONPC (8 mM) as a substrate 45°C in 50 mM sodium acetate buffer, pH 5.5.

Reversibilität

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Photoresponsive polymer-enzyme switches

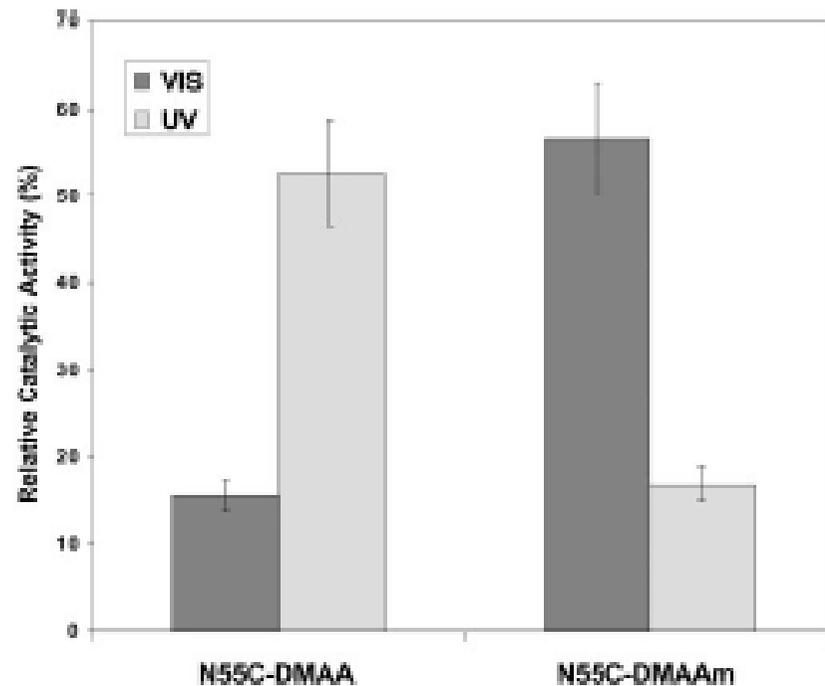


Fig. 5. Photoinduced activity changes of N55C-DMAA and N55C-DMAAm conjugates when immobilized on streptavidin-coated magnetic beads. Conditions are described in Materials and Methods.

Immobilisation an magnetic beads

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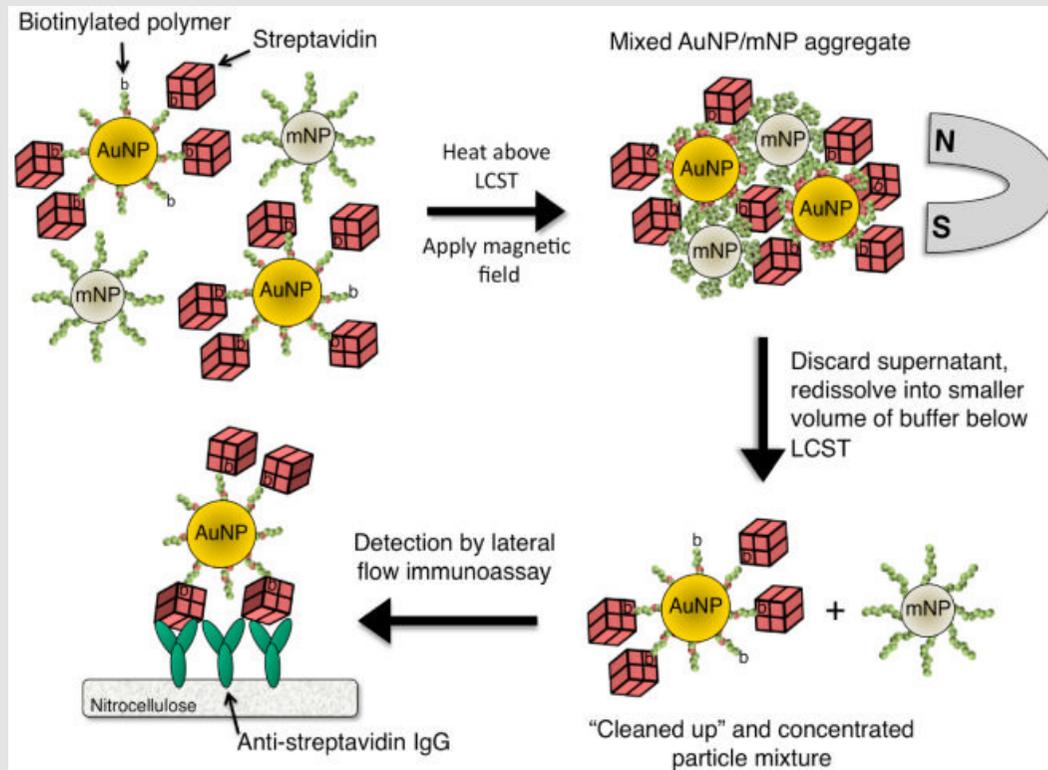
2. Photoresponsive polymer-enzyme switches

Fazit:

- Enzyme konnten mithilfe der Polymere durch Lichtbestrahlung an und aus geschaltet werden
 - > Kontrolle der Enzymaktivität
 - > Enzym kann ausgeschaltet bleiben bis es im Körper sein Ziel erreicht und dort mithilfe von optischer Fibertechnologie angeschaltet werden
- Reversibler Prozess
 - Microfluide
 - Lab on a chip

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3. Ausblick



Weitere Anwendungen

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4. Quellen

Quellen:

- **Photoresponsive polymer–enzyme switches**

Tsuyoshi Shimoboji*, Edmund Larenas†,

Tim Fowler‡, Samarth Kulkarni*, Allan S. Hoffman*‡, and Patrick S. Stayton*‡

- **Really smart bioconjugates of smart polymers and receptor proteins**

Allan S. Hoffman,1* Patrick S. Stayton,1 Volga Bulmus,1 Guohua Chen,1 Jingping Chen,1 Chuck Cheung,1

Ashutosh Chilkoti,1 Zhongli Ding,1 Liangchang Dong,1 Robin Fong,1 Chantal A. Lackey,1 Cynthia J. Long,1

Morikazu Miura,1 John E. Morris,1 Niren Murthy,1 Yoshikuni Nabeshima,1 Tae Gwan Park,1

Ollie W. Press,1 Tsuyoshi Shimoboji,1 Sara Shoemaker,1 Heung Joon Yang,1 Nobuo Monji,2

Robert C. Nowinski,2 Carole Ann Cole,2 John H. Priest,2 J. Milton Harris,3 Katsuhiko Nakamae,4

Takashi Nishino,4 Takashi Miyata4

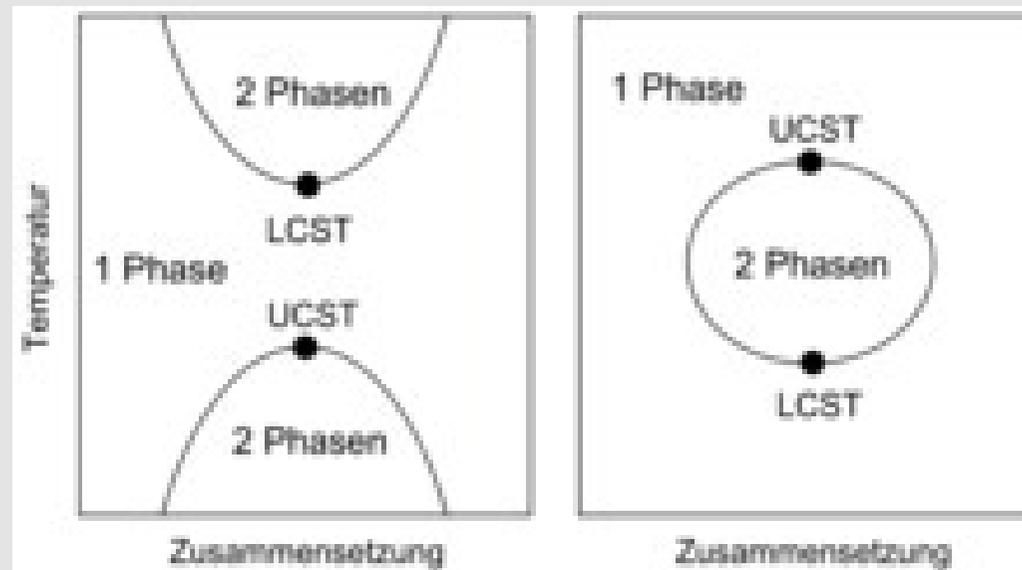
- **A Mixed Stimuli-Responsive Magnetic and Gold Nanoparticle System for Rapid Purification, Enrichment, and Detection of Biomarkers**

Michael A. Nash, Paul Yager, Allan S. Hoffman, and Patrick S. Stayton*

Department of Bioengineering, University of Washington, Seattle, WA 98195

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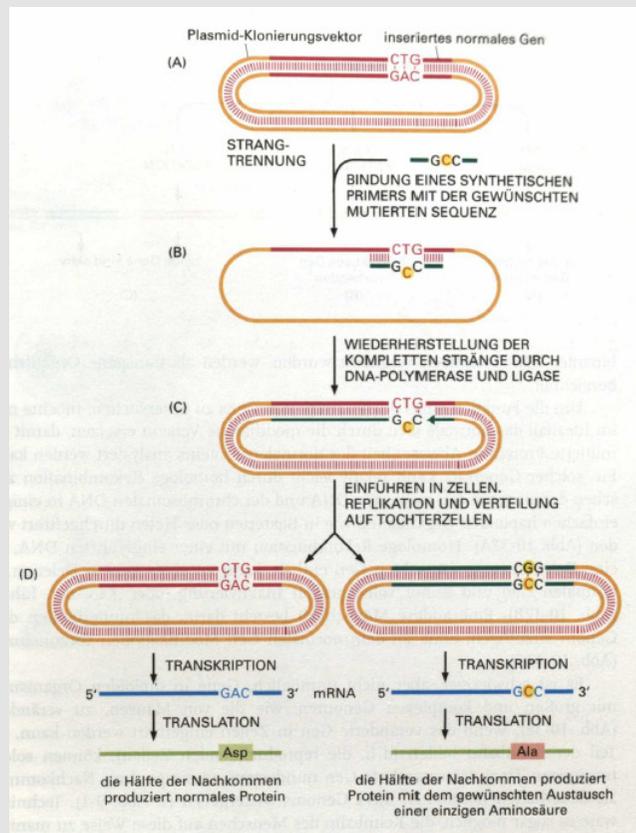
1. Einführung in die Technik



Lower critical solution
Temperature = LCST

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2. Photoresponsive polymer-enzyme switches



Ortsspezifische Mutagenese

- gezielte Steuerung der Bindung des Polymers an das Enzym