

Surface Display als Werkzeug in der Analytik und der evolutiven Entwicklung von Biologicals

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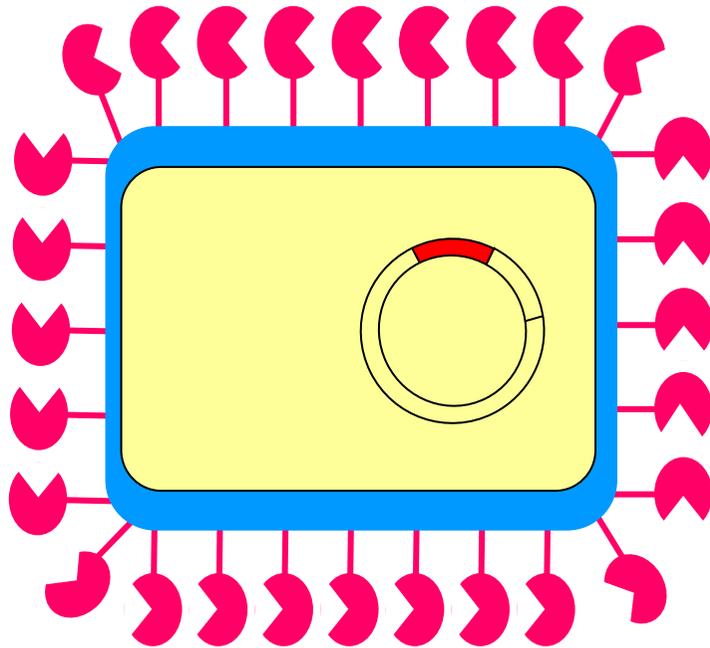
Surface Display als Werkzeug in der Analytik und der evolutiven Entwicklung von Biologicals

Einleitung

1. **Surface Display – das Autodisplay System**
2. **Expression und Screening von Peptidbibliotheken**
3. **Display und Analytik von Rezeptoren**
4. **Evolute Entwicklung von Biologicals am Beispiel von Antikörperfragmenten**
5. **ELISA Entwicklung**

Zusammenfassung und Ausblick

Surface Display

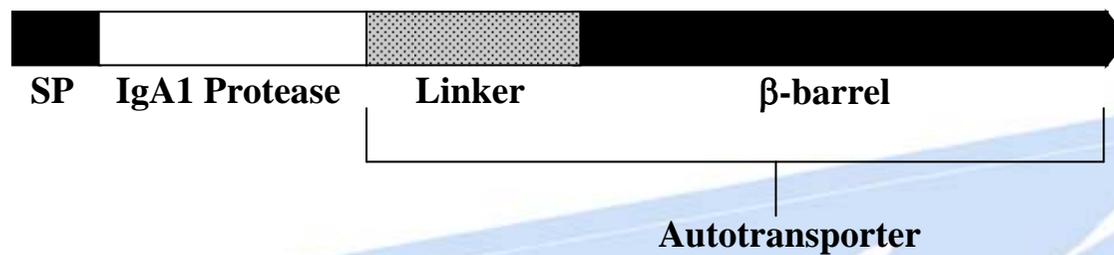
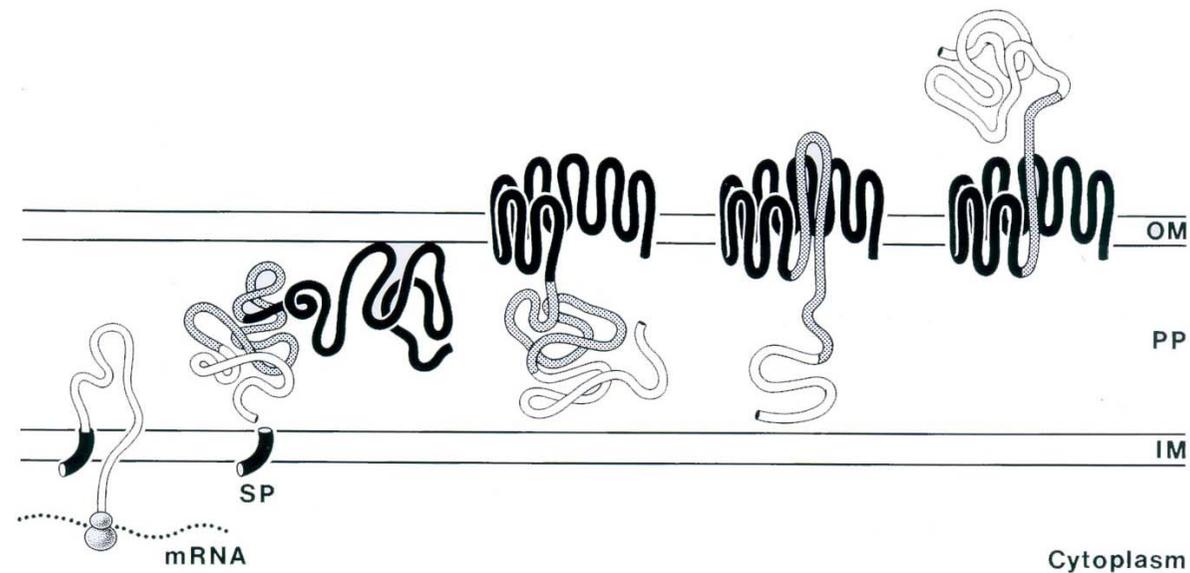


Vorteile:

- Moleküle frei zugänglich
- immobilisiert und stabilisiert
- einfache Aufreinigung
- internes Label: DNA

- selbstreplikativ
- mit optischen Methoden z.B. FACS analysierbar

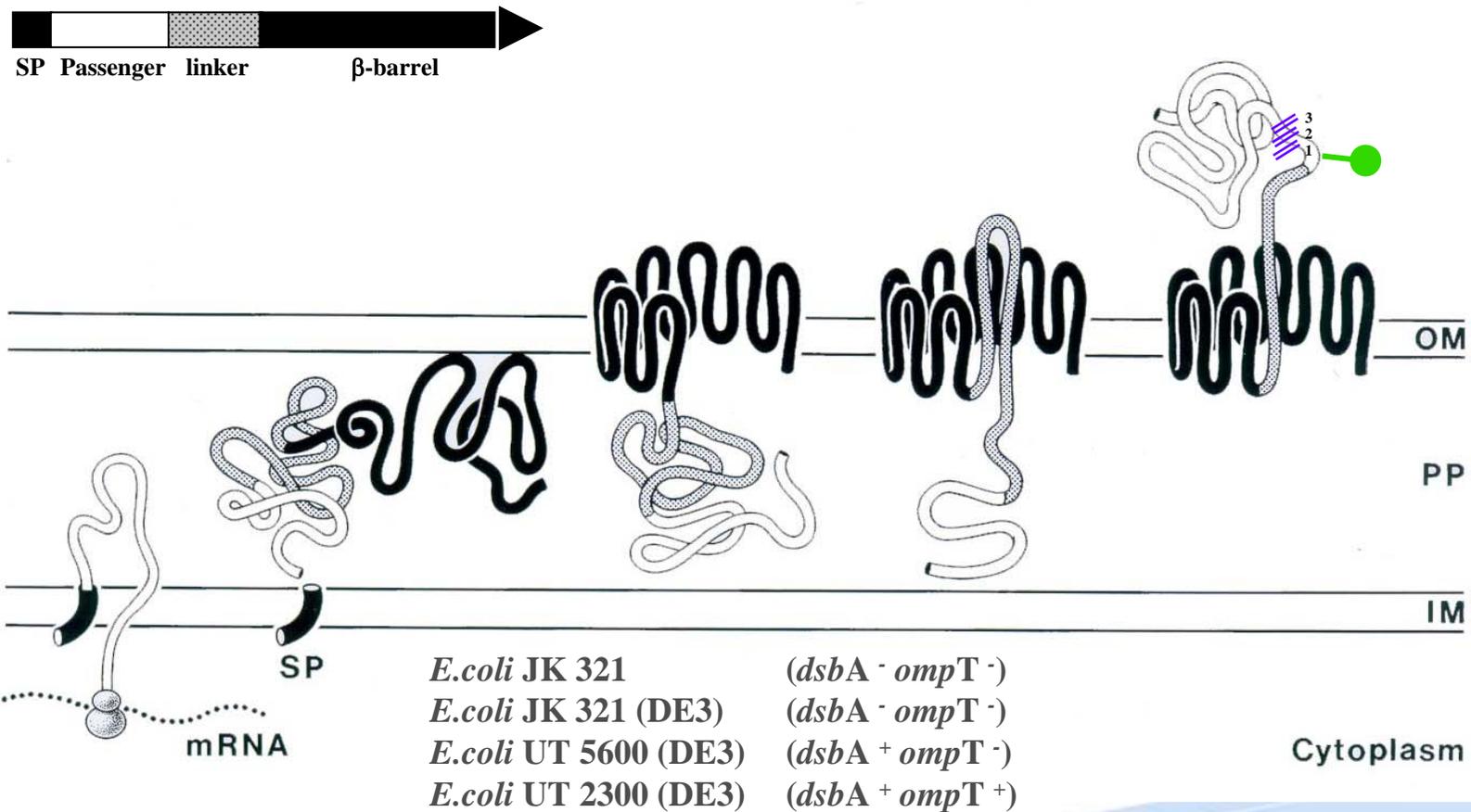
Autotransporter - Sekretionsmechanismus



Mol Microbiol (1995), 18: 380-382.

Autodisplay

„Cystope tagging“



Gene (1996), 178:107-110

J Bacteriol (1997), 179:794-804

J Bacteriol (1999), 181:7014-7020

ChemBioChem (2003), 4:396-405

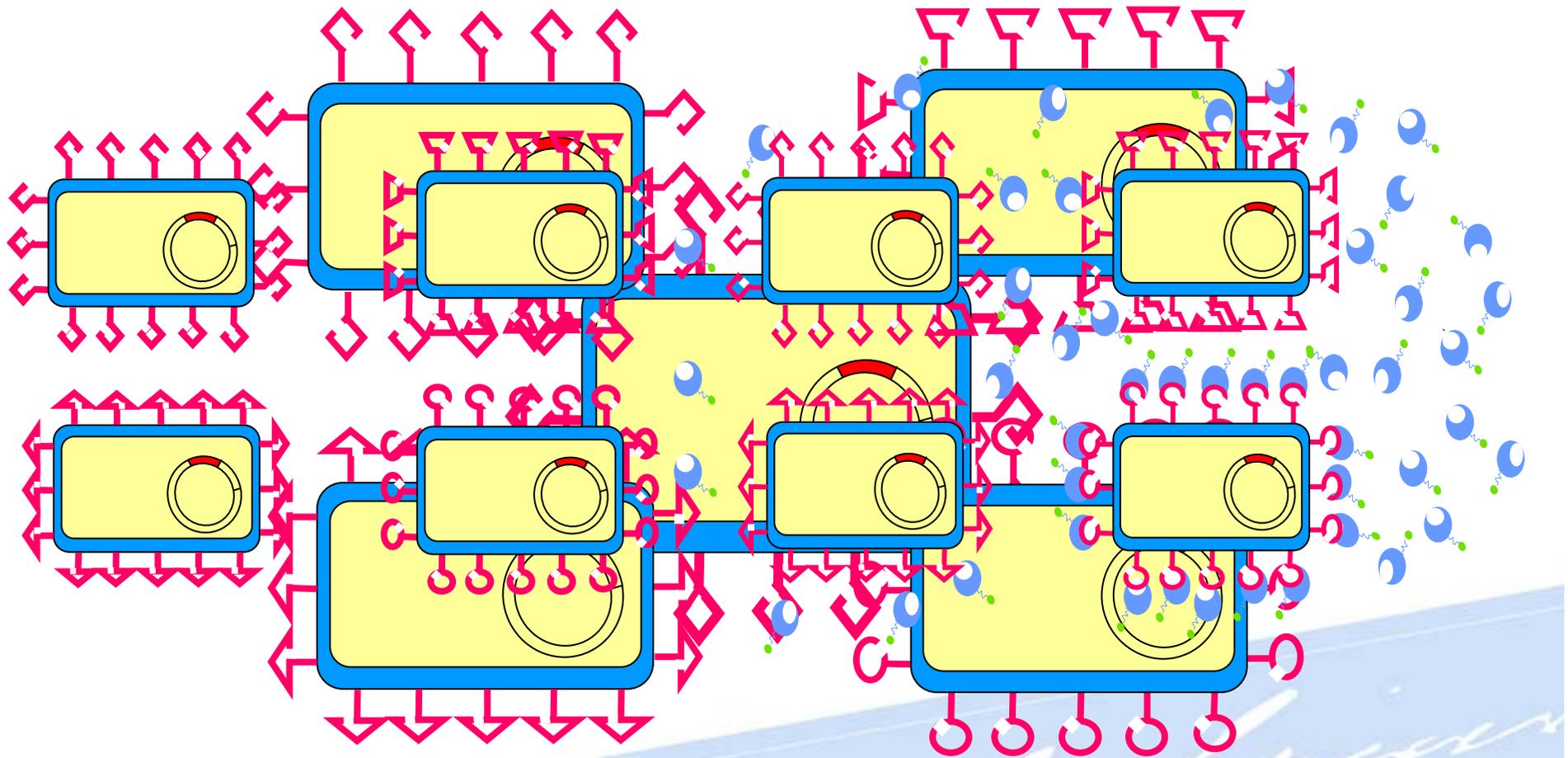
Anal Biochem (2004), 331:267-274

Appl Microbiol Biotechnol (2006), 69:607-614

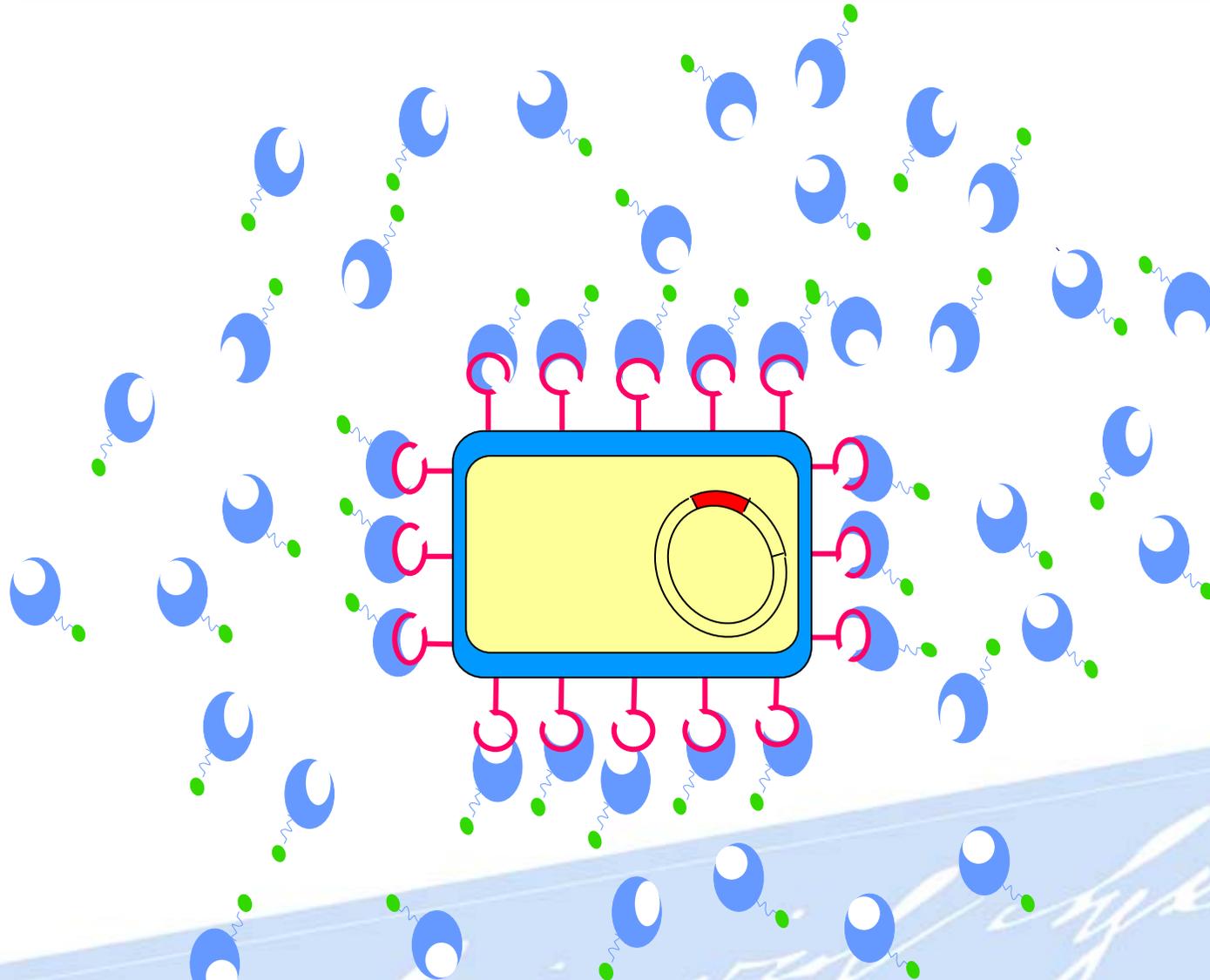
Microbiol Mol Biol R (2007), 71:600-619

Appl Environ Microbiol (2008), 74:4782-4791

Expression von Peptidbibliotheken



Expression von Peptidbibliotheken



Evolute Entwicklung von Enzyminhibitoren

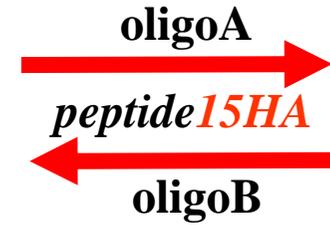
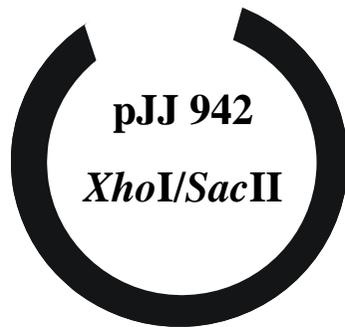
<u>Target</u>	<u>Indikation</u>	<u>Leitstruktur</u>
Cathepsin G	Lungenemphysem	P15
Leukozyten Elastase	Rheumatoide Arthritis Lungenemphysem	Aprotinin, P15
CK2 (Hyal-1, -2 Spam-1)	Zellproliferation Krebs	<i>kinA, kinS</i>

Protease Inhibitor Ausgangsstruktur

C- reaktives Protein (CRP), wird im Verlauf einer akuten Entzündung im Blut akkumuliert, Entzündungsmarker

Peptid15 (P15) abgeleitet von CRP ist ein potenter Inhibitor von humaner Leukozyten Elastase (HLE; $K_i = 0,18 \mu\text{M}$) und humanem Cathepsin G (HCG; $K_i = 0,25 \mu\text{M}$)

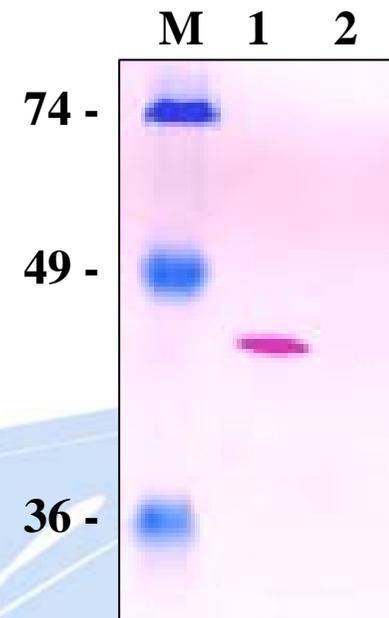
Autodisplay von P15



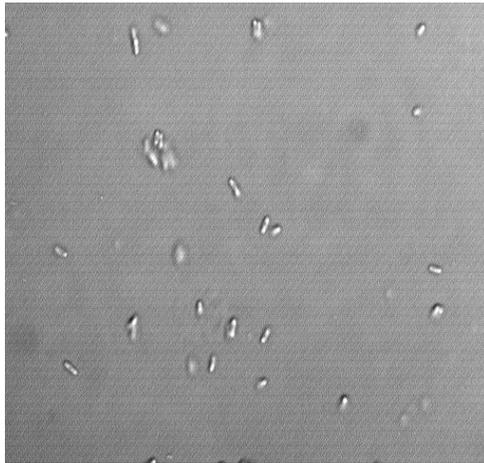
ligation



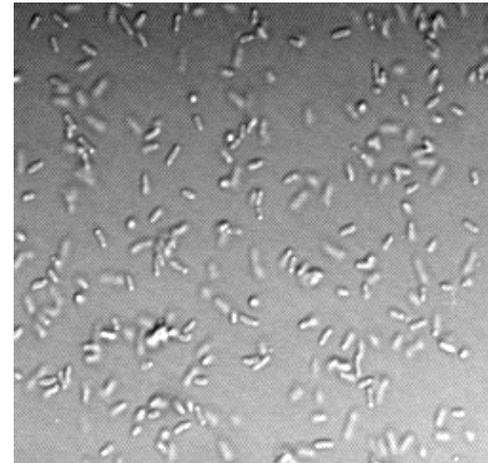
Anal Biochem (2005), 346:258-267



Nachweis der Oberflächenständigkeit



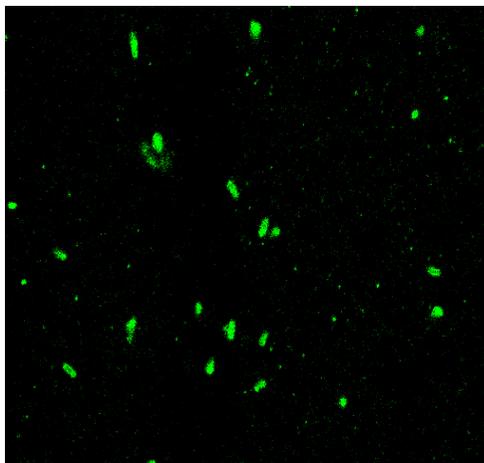
Transmission



UT560/pDZ15HA

UT560/pJJ942

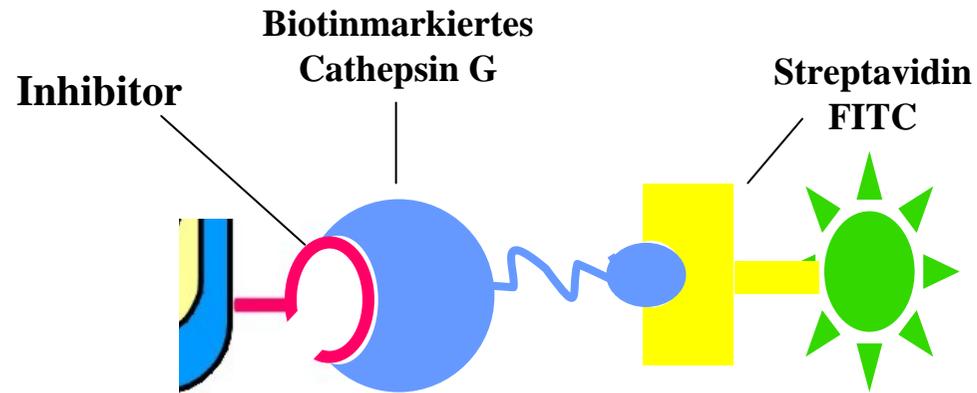
Anal Biochem (2005),
346:258-267



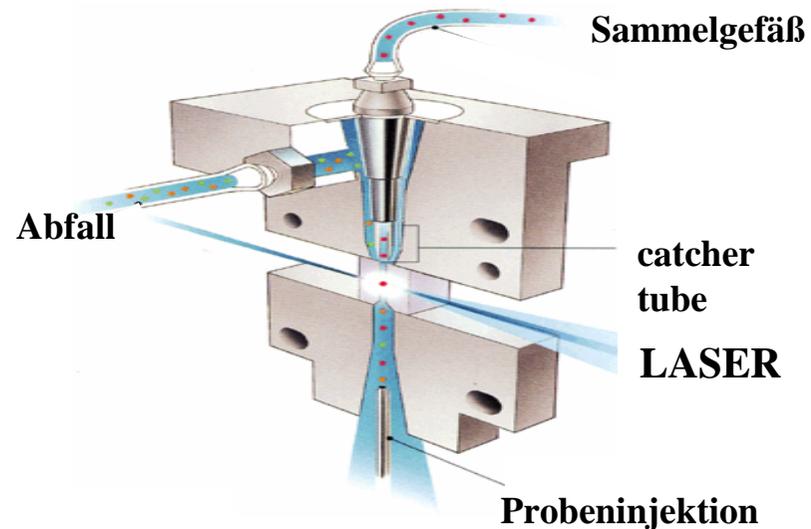
Fluoreszenz



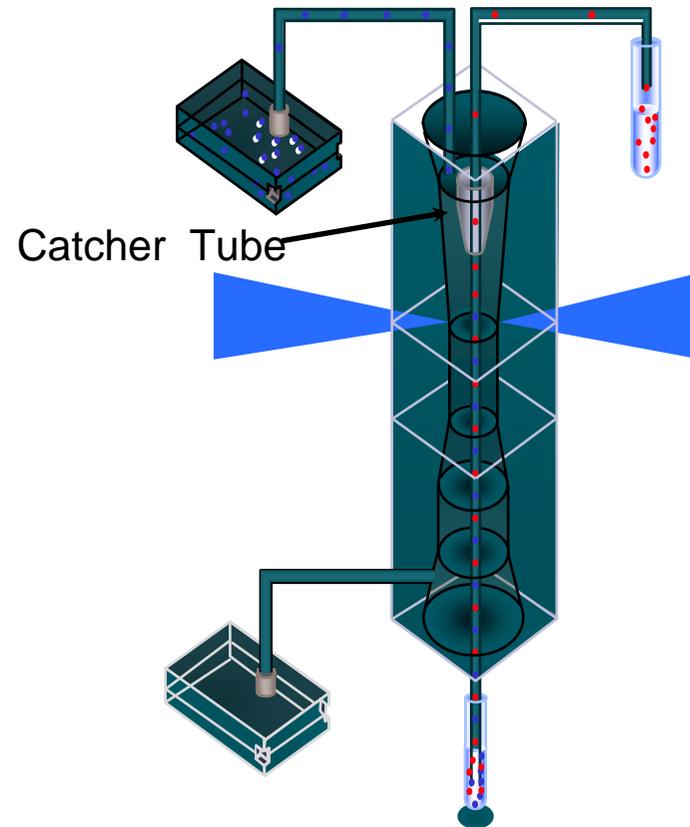
Enzymbindung und Durchflußzytometrie



*Anal Biochem (2005),
346:258-267*

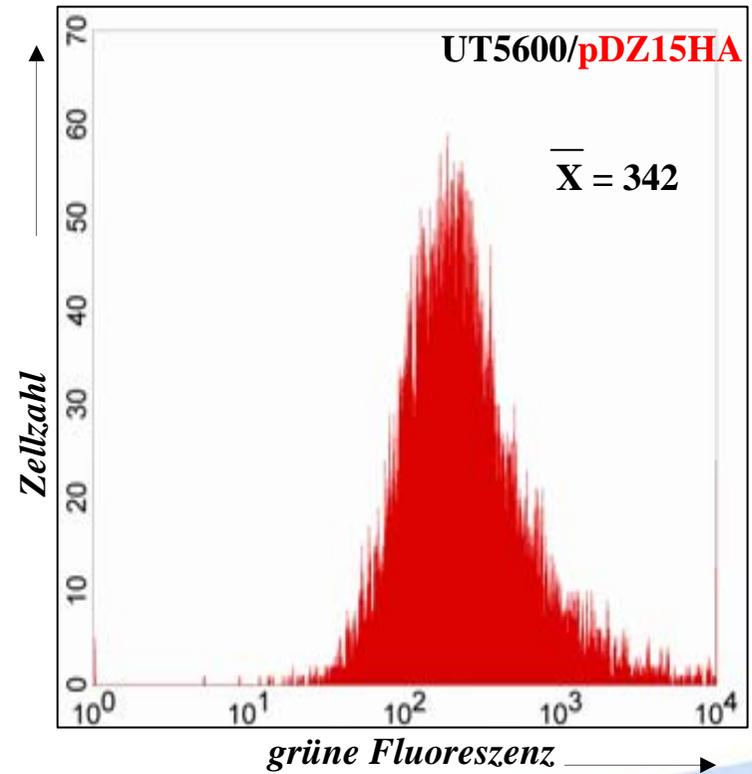
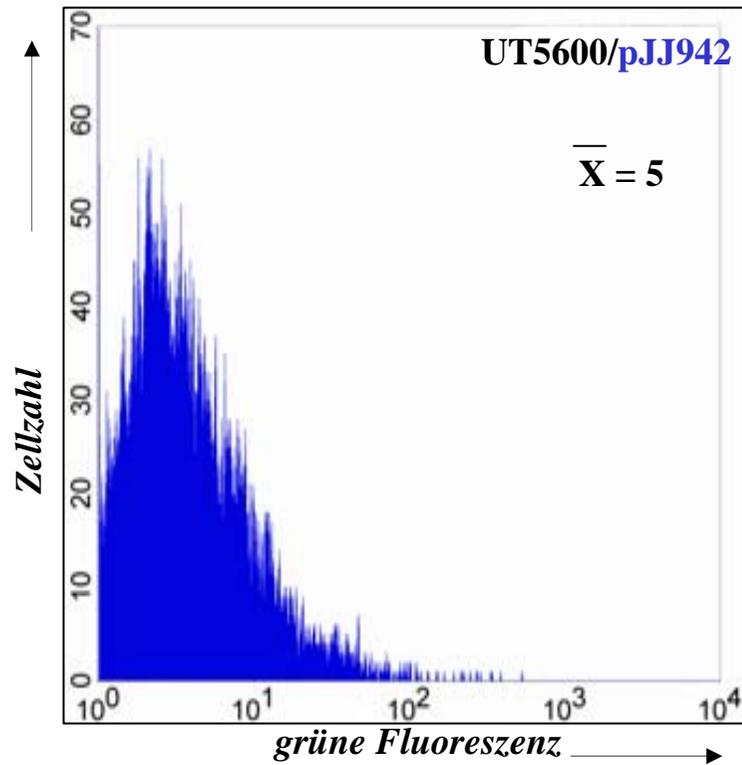


Enzymbindung und Durchflußzytometrie



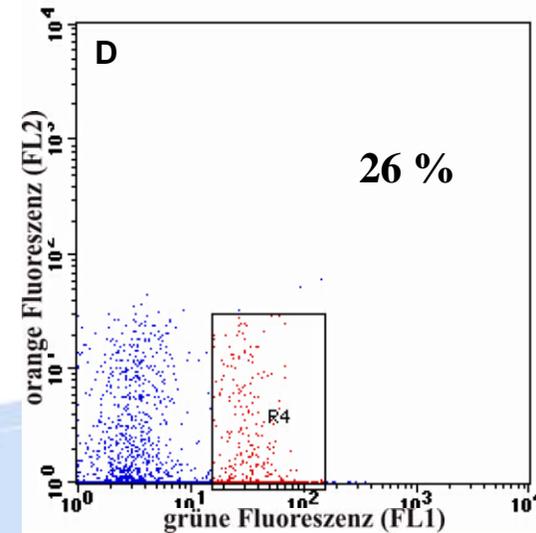
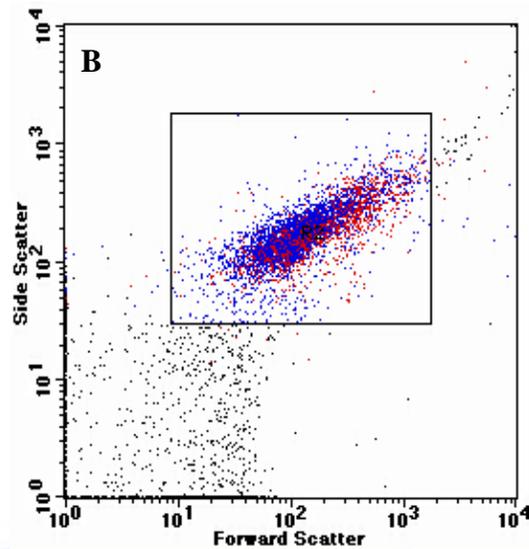
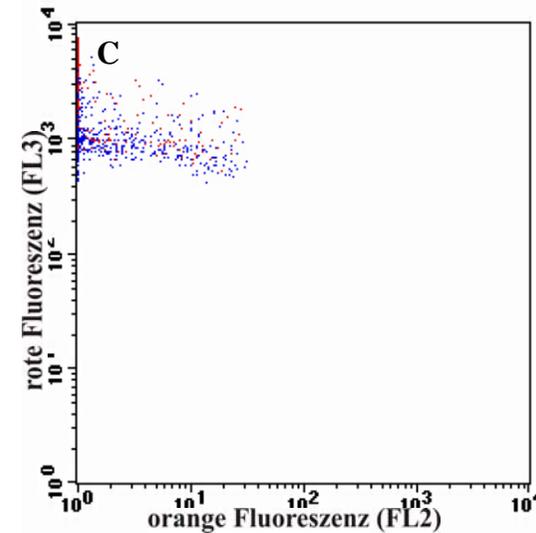
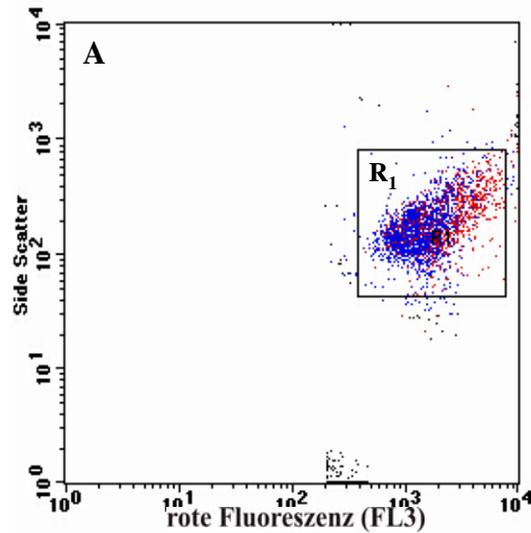
FACS – Einzelzellanalyse

Fluorescence Activated Cell Sorting



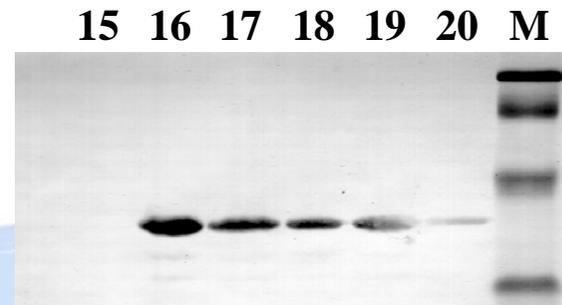
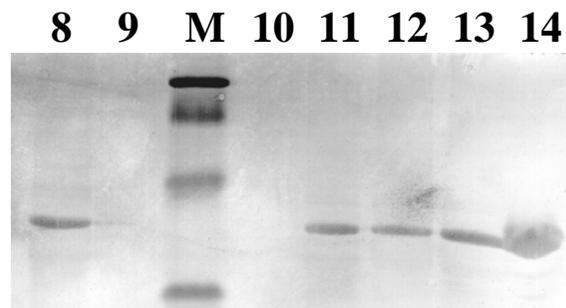
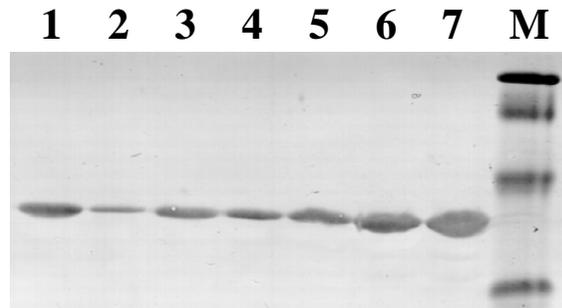
Anal Biochem (2005),
346:258-267

FACS – Einzelsortierung



Anal Biochem (2005),
346:258-267

Analyse der Zellklone



Anal Biochem (2005),
346:258-267

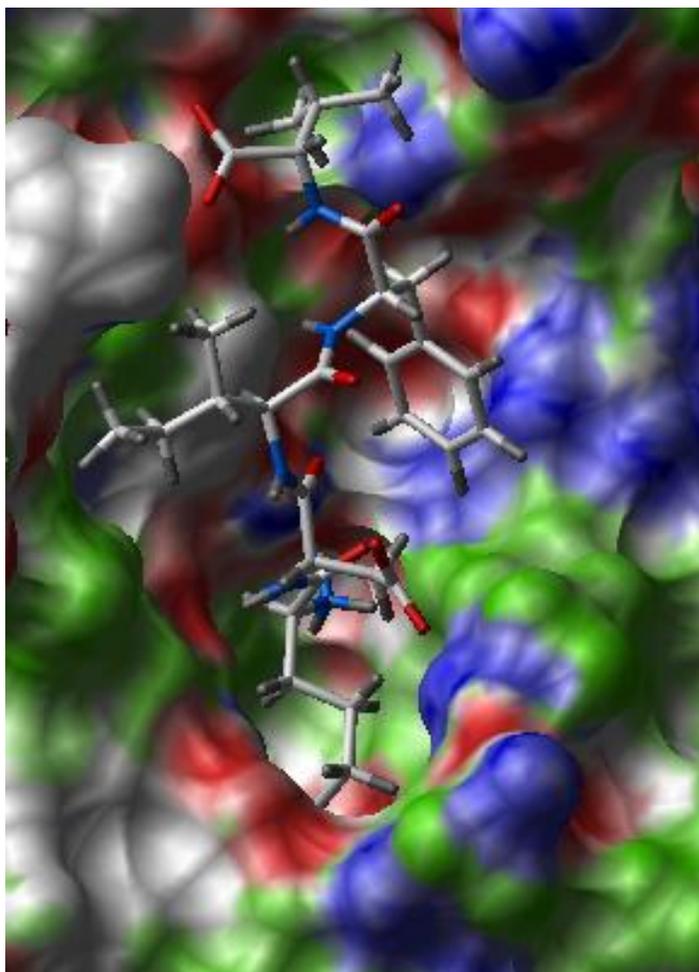
Neue Cathepsin G Inhibitoren

		% Hemmung (50 μ M)	IC ₅₀ ¹⁾
P3	RNTQHTNIYAKDIFV	50,0	
P4	GNTQHTNIYAKDIFV	53,2	
P5	GIPQHTNIYAKDIFV	49,4	
P6			
	KDIFV	71,2	11,7 μ M
P7			
	KDIF	25,9	
P15	EILIFWSKDIGYSFT	>90,0	4,5 μ M

¹⁾ bei 22° C

Anal Biochem (2005),
346:258-267

Rationale Optimierung

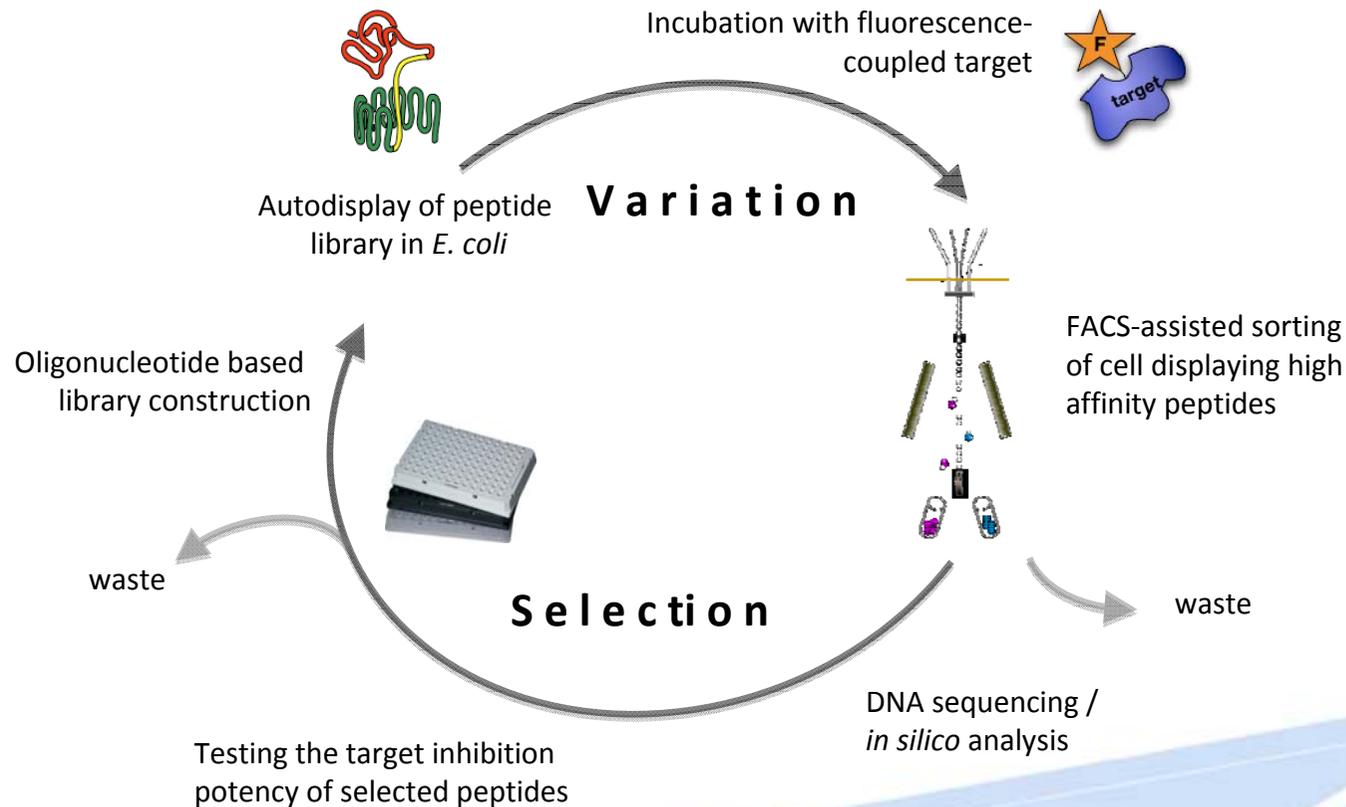


name	IC ₅₀ ¹⁾ (μM)	K _i ²⁾ (μM)
P15	8,08	0,98
P6	19,56	2,37
P6a	9,76	1,18
P6b	12,87	1,56

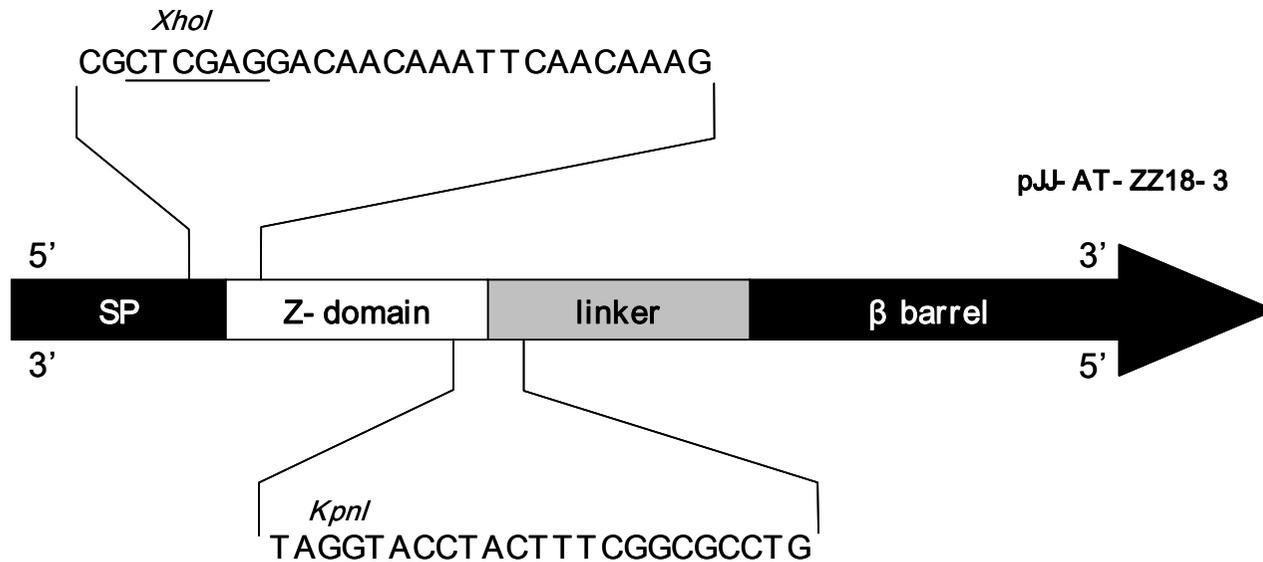
¹⁾ bei 37° C

²⁾ K_m nach Yavin et al. 1996

Evolutionary Drug Development



Autodisplay der Z Domäne von Protein A



Biosens Bioelectron, (2008)
doi:10.1016/j.bios.2008.07.067.

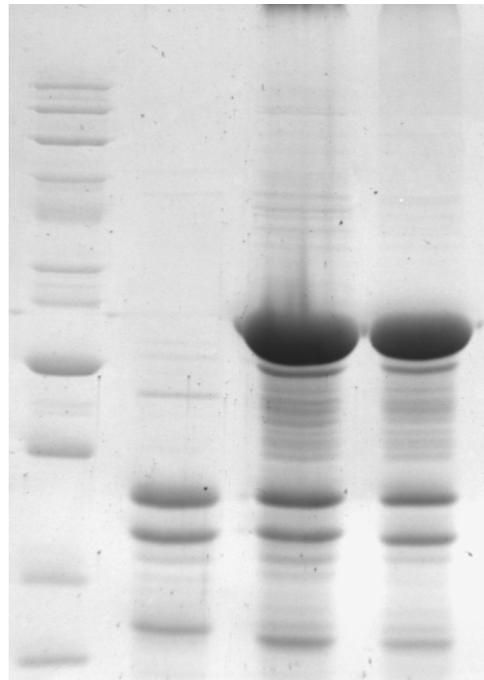
Autodisplay der Z Domäne von Protein A

Marker 1 2 3

1: nicht induziert

2: induziert, ohne DTT

3: induziert, mit DTT



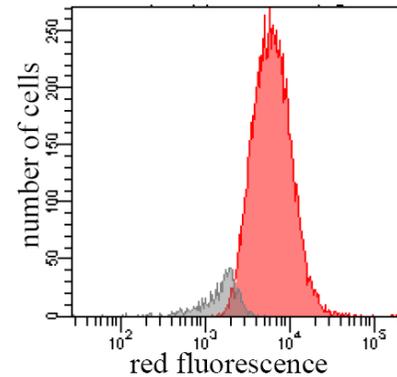
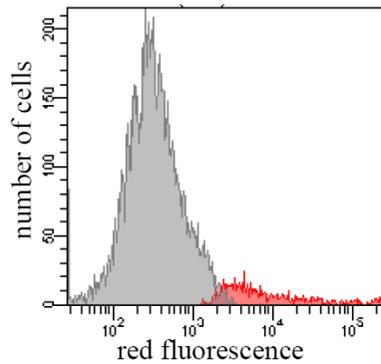
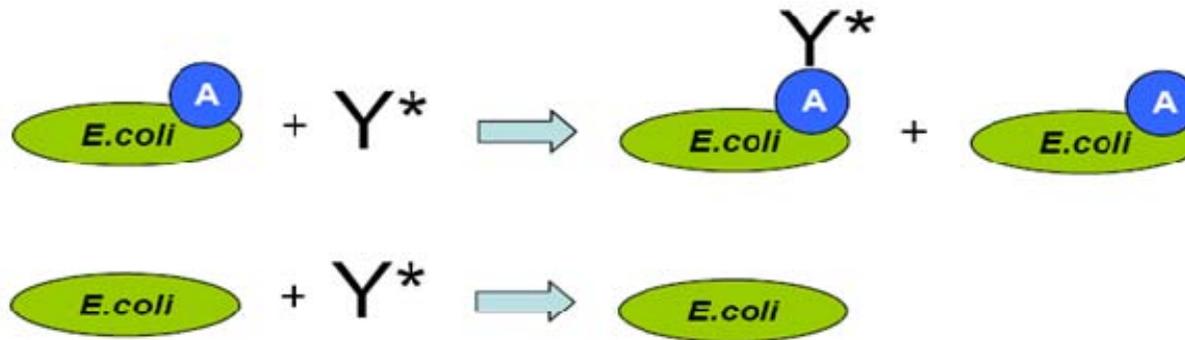
← Z Domäne

← OmpF/C

← OmpA

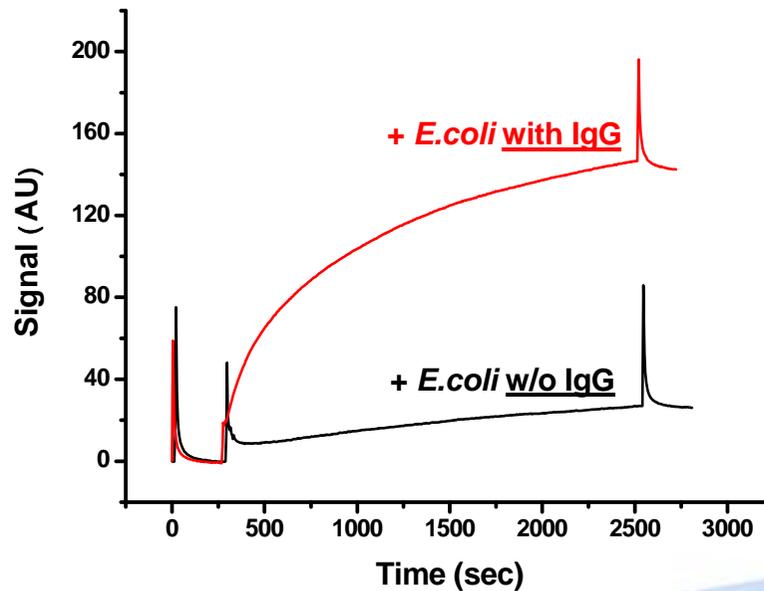
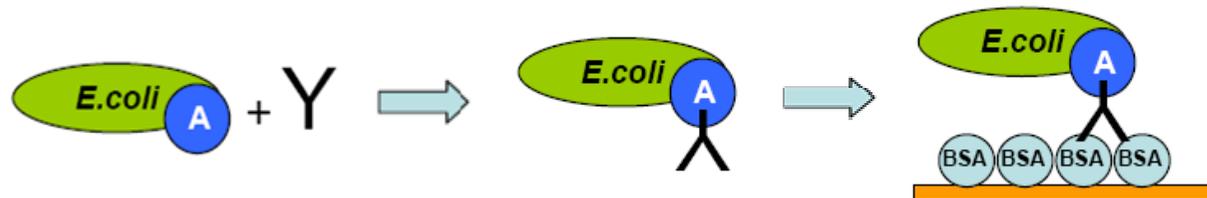
Biosens Bioelectron, (2008)
doi:10.1016/j.bios.2008.07.067.

FACS Nachweis der IgG-Bindung



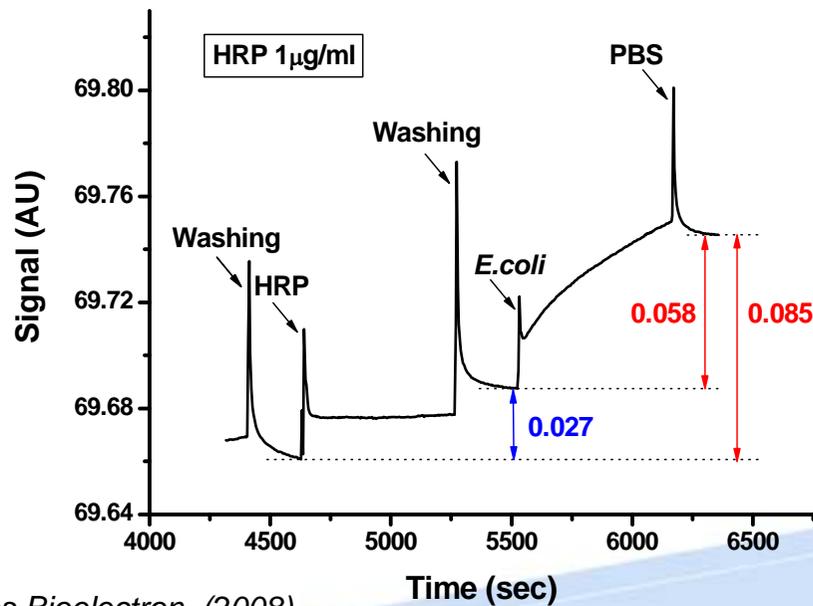
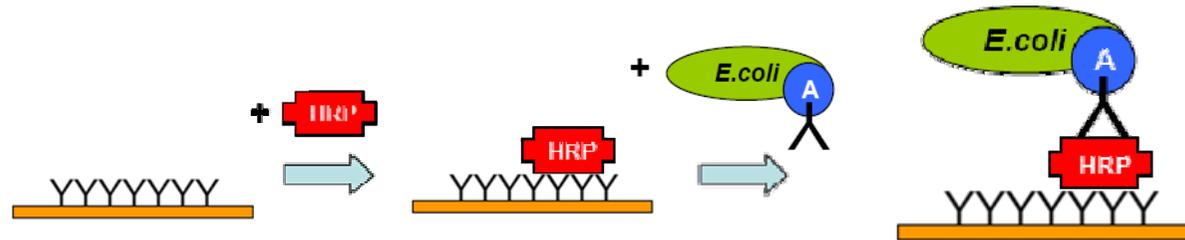
Biosens Bioelectron, (2008)
doi:10.1016/j.bios.2008.07.067.

Signalamplifikation in der SPR



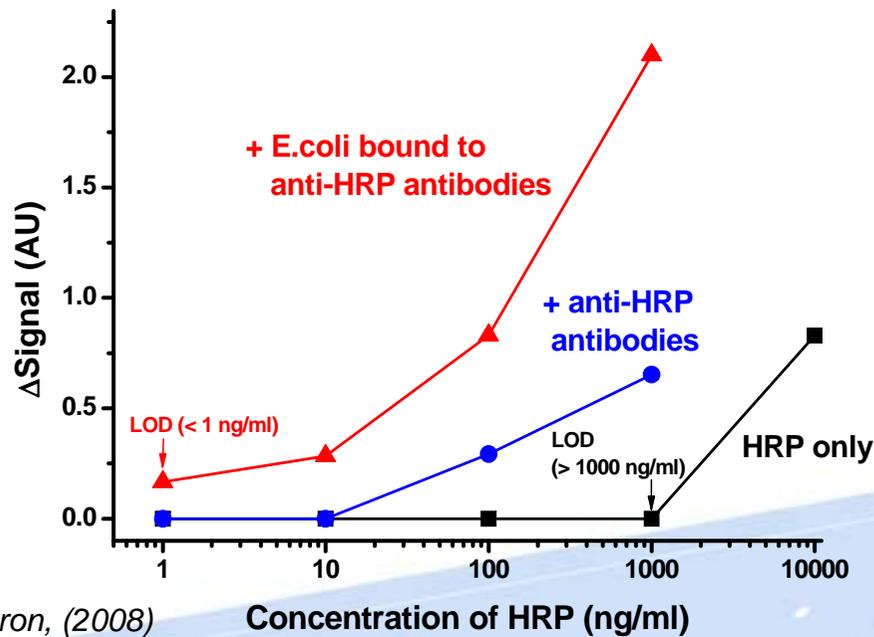
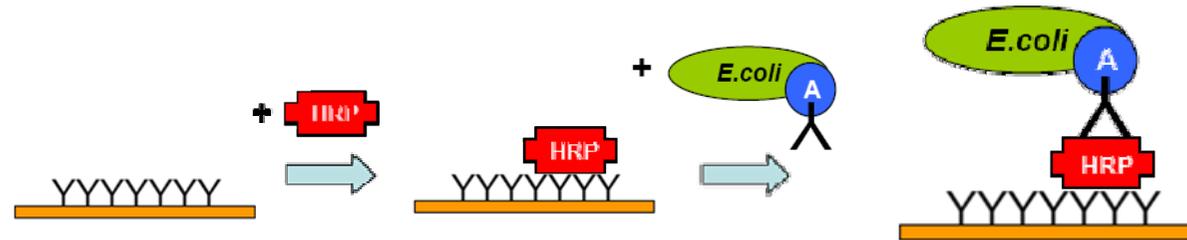
Biosens Bioelectron, (2008)
doi:10.1016/j.bios.2008.07.067.

Signalamplifikation in der SPR



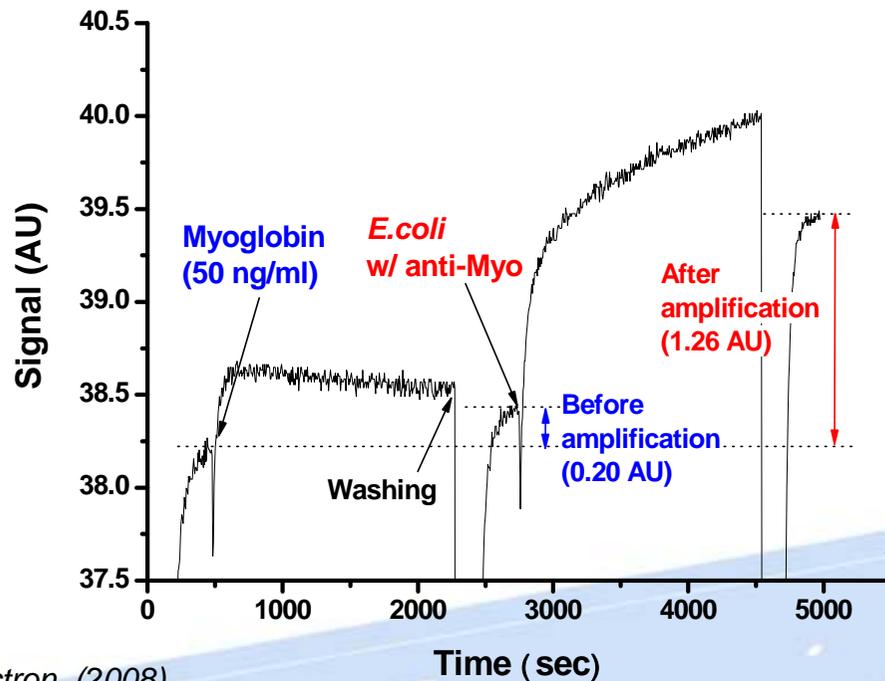
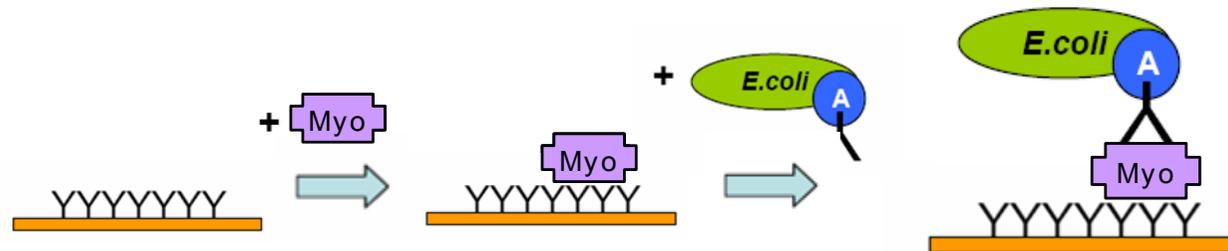
Biosens Bioelectron, (2008)
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Signalamplifikation in der SPR



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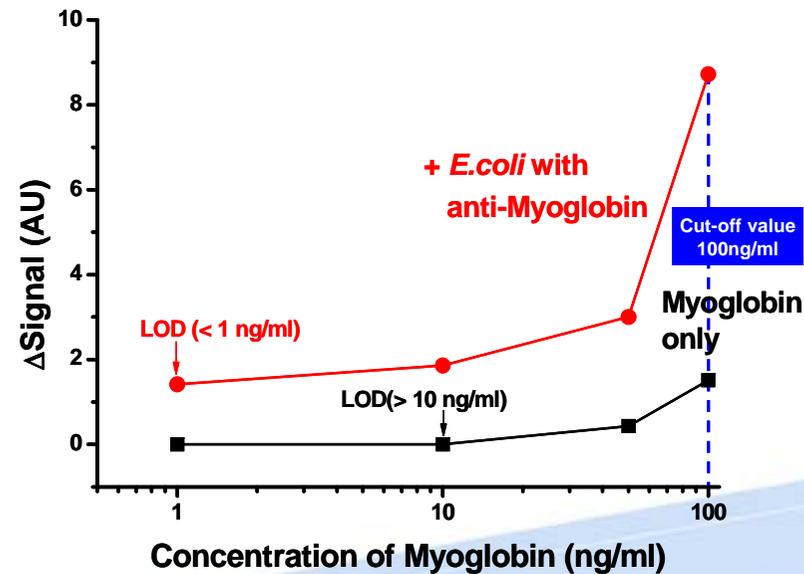
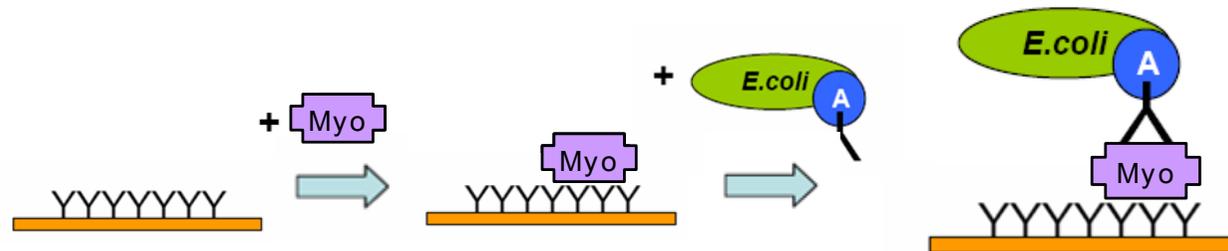
Signalamplifikation in der SPR



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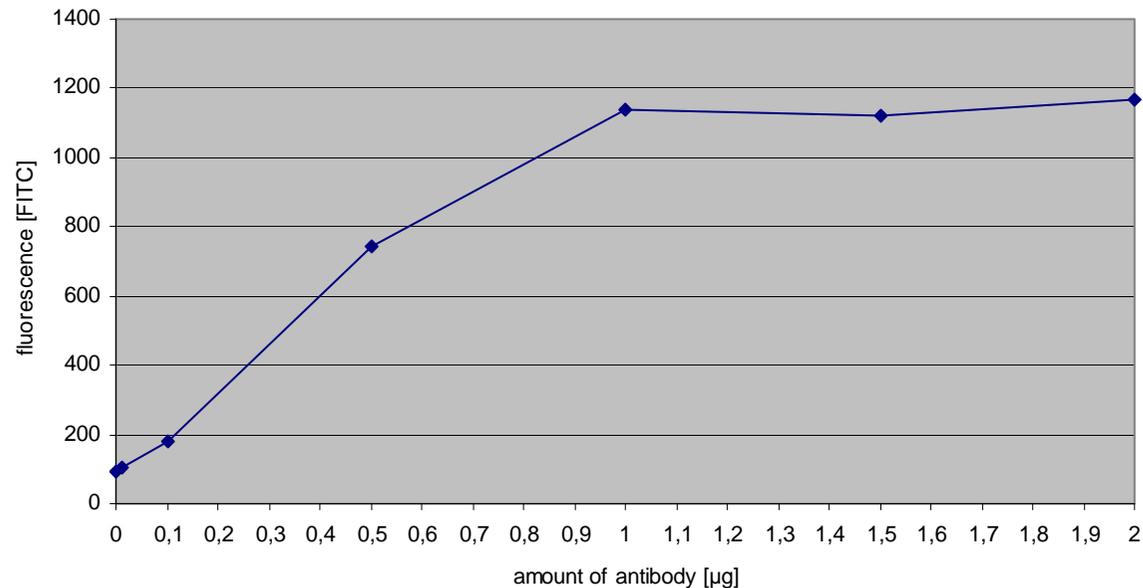
Symposium der Fachgruppe Arzneimittelkontrolle/Pharmazeutische Analytik, Bonn, 10.-11.10.08

Signalamplifikation in der SPR



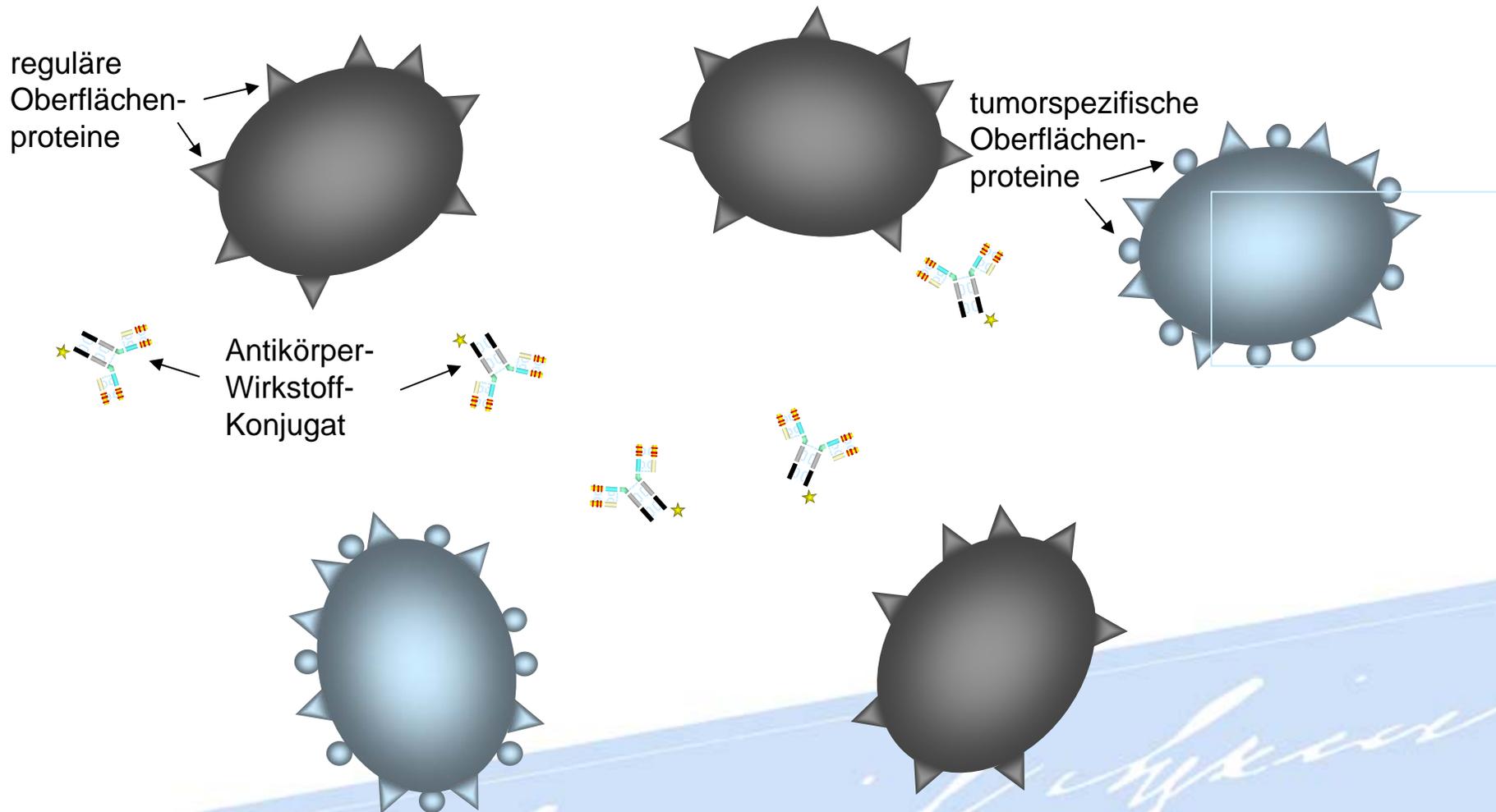
Biosens Bioelectron, (2008)
doi:10.1016/j.bios.2008.07.067.

Z Domänen/Zelle bestimmt durch IgG-Bindung und FACS

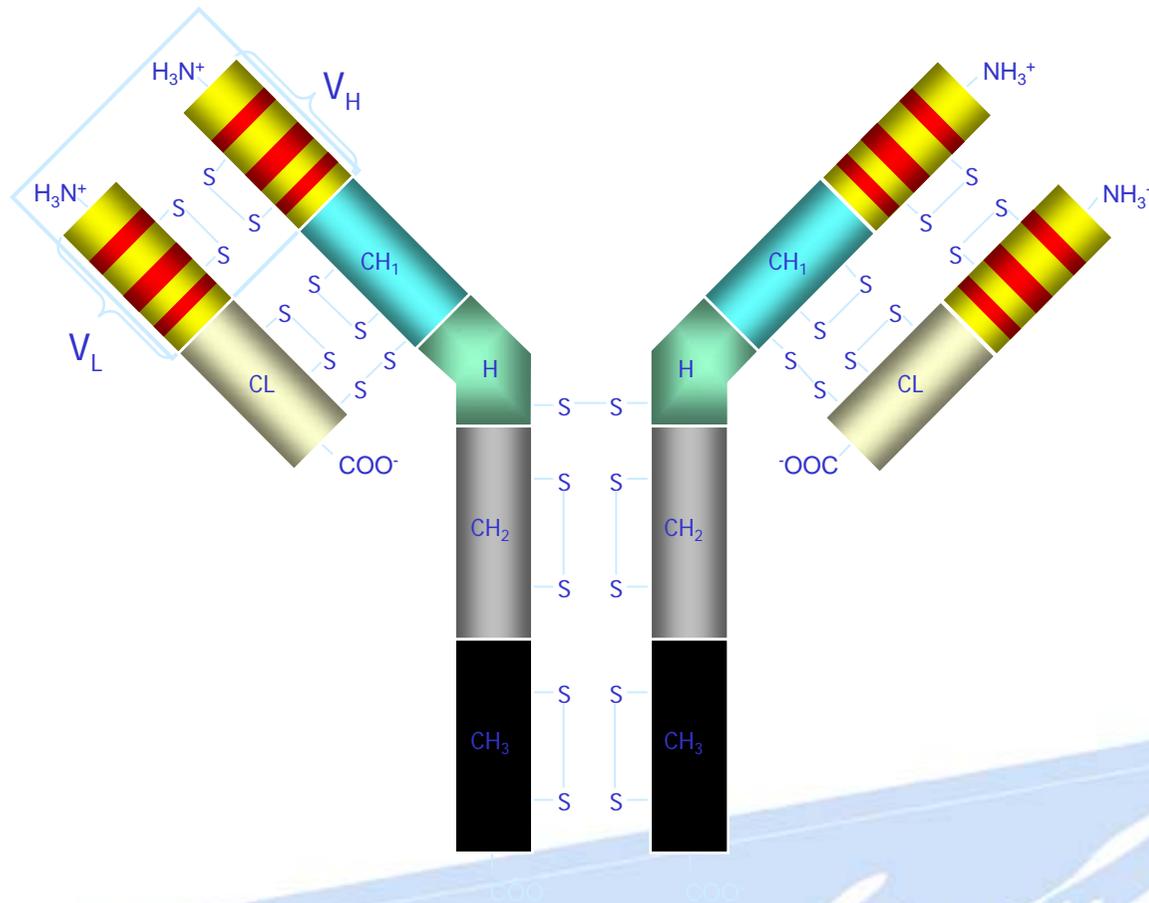


⇒ 225.000 Bindungsstellen/CFU

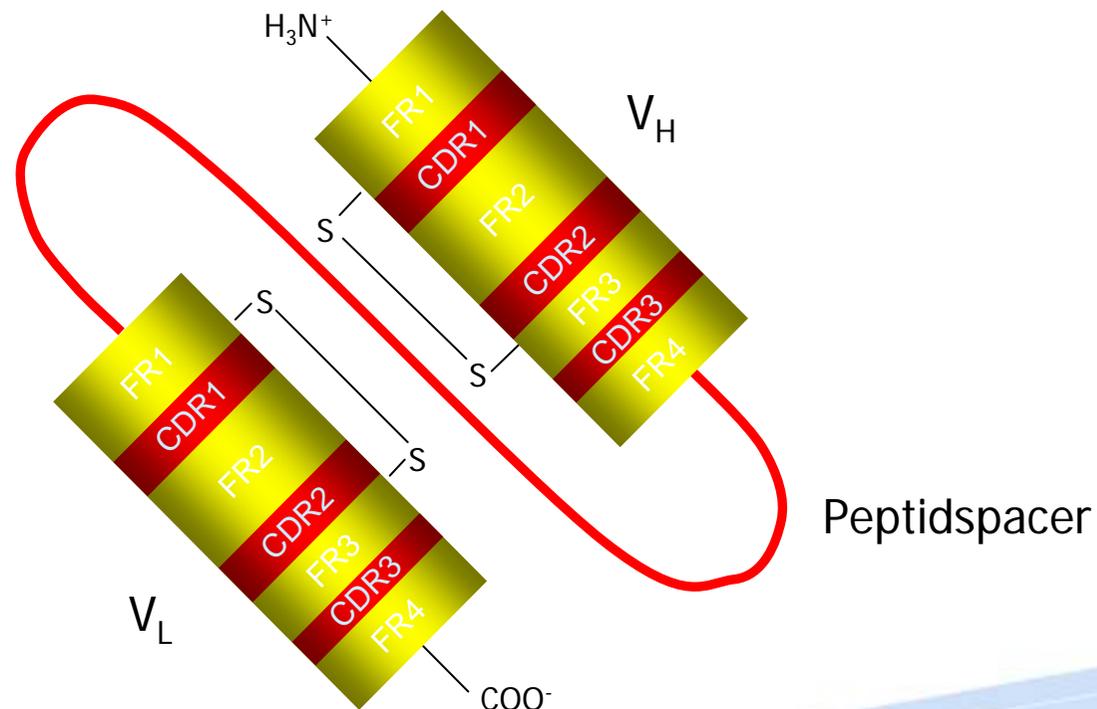
Evolutives Entwicklung von Antikörperfragmenten (Biologicals)



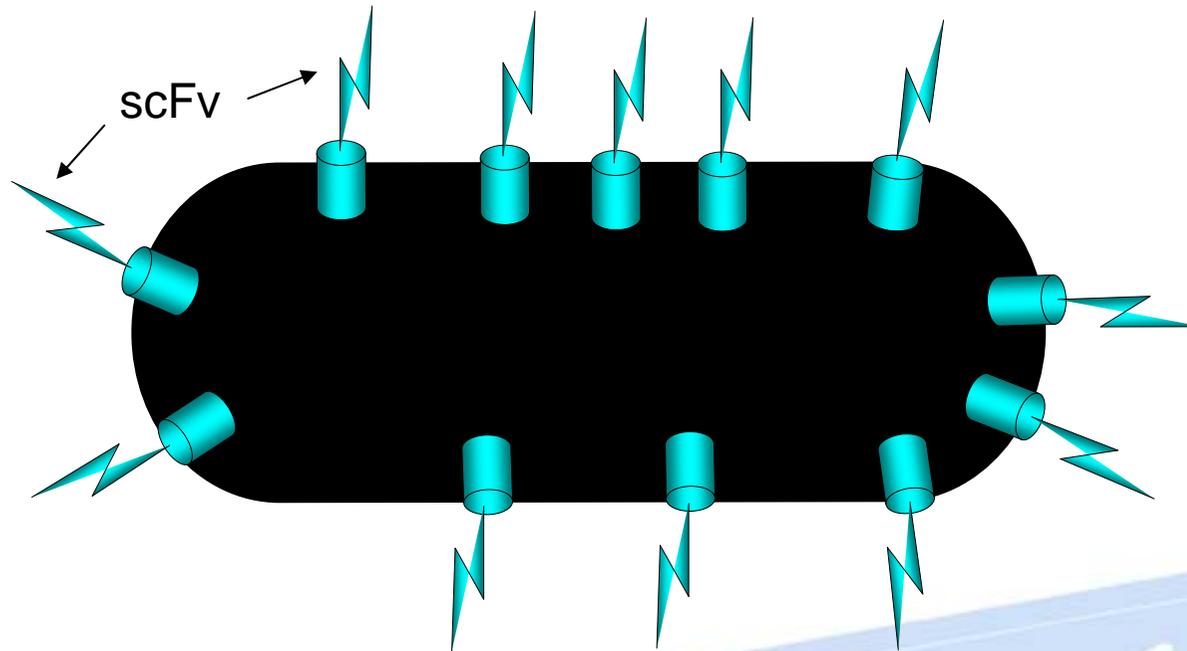
Aufbau eines Antikörpers



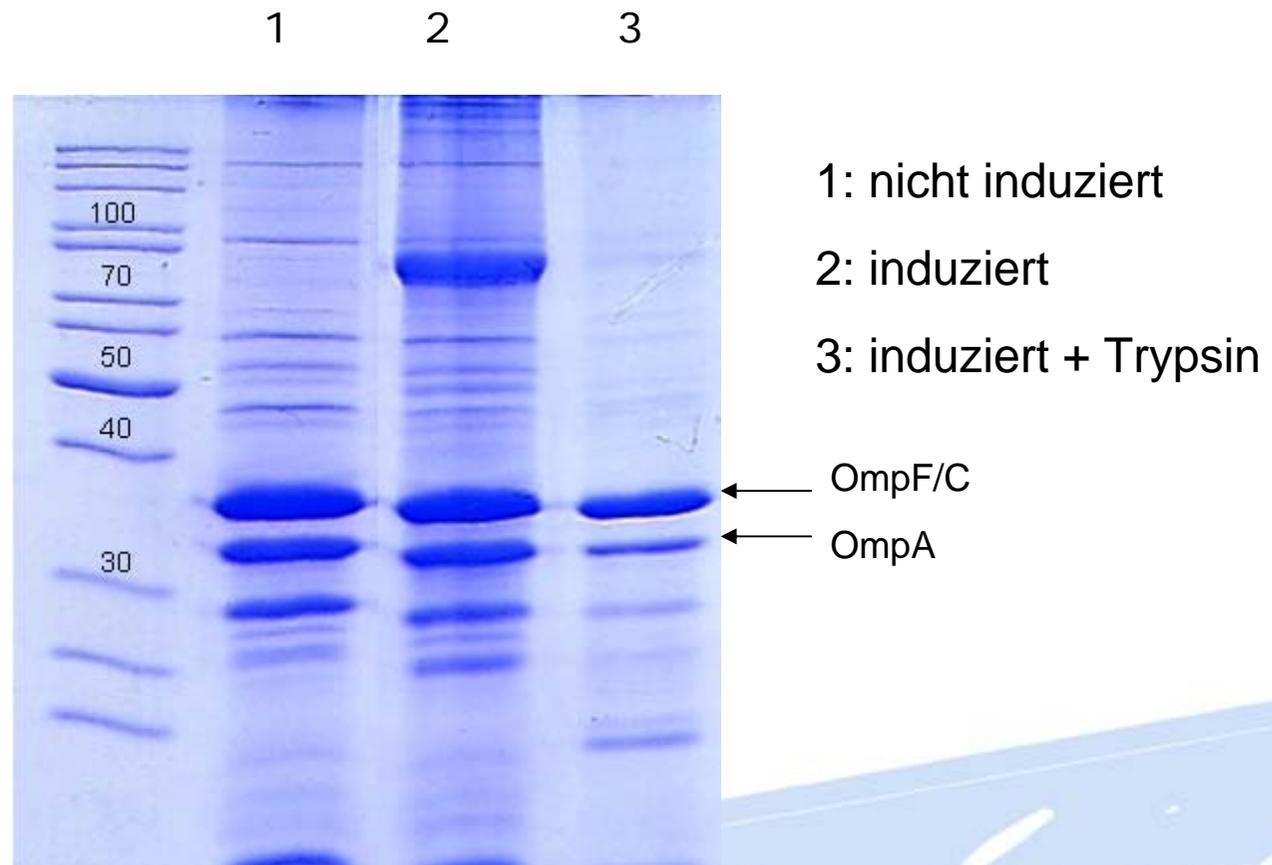
Reduzierte Wirkform ("single chain", scFv)



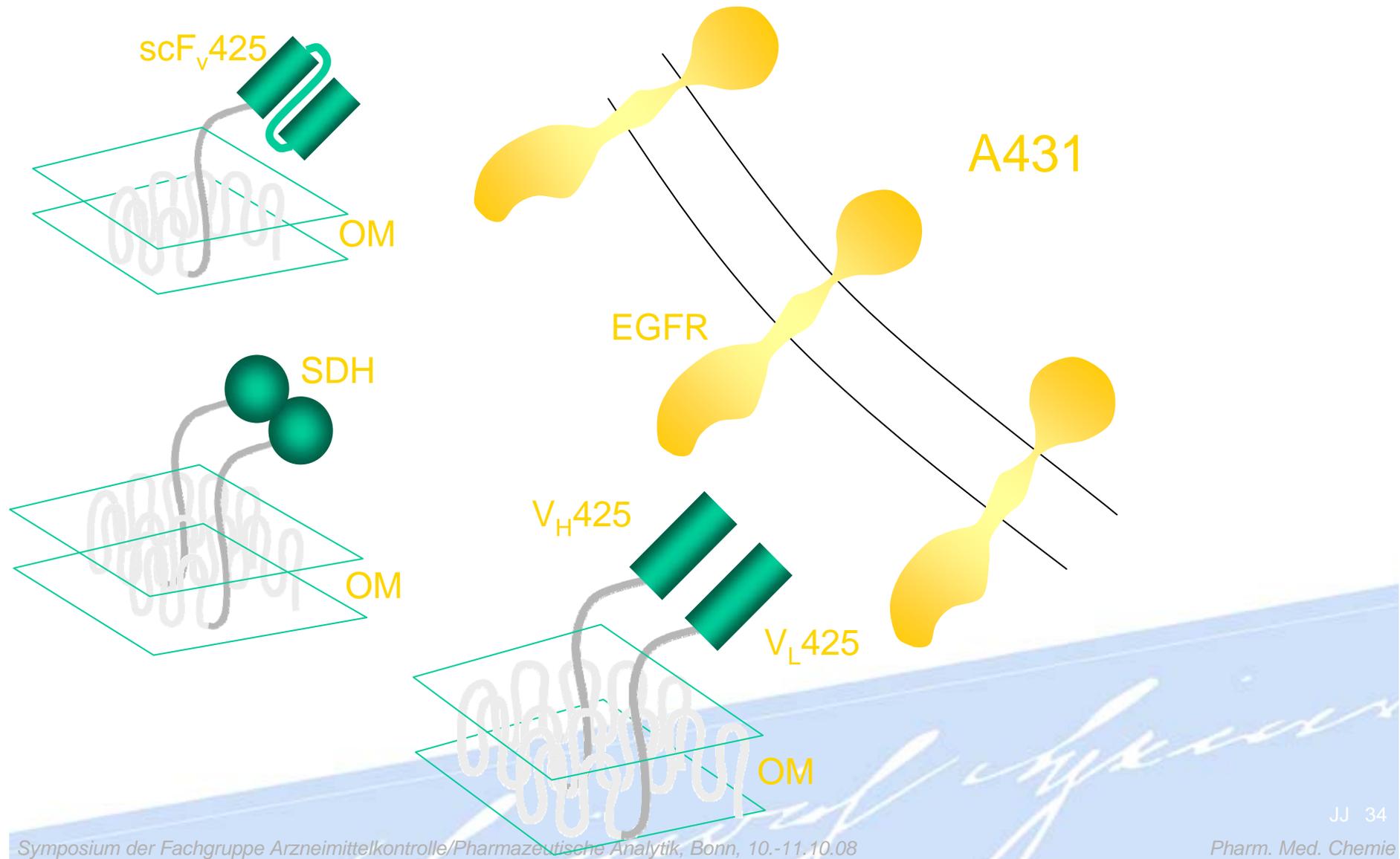
Autodisplay von scFv



Autodisplay eines scFv gegen EGFR



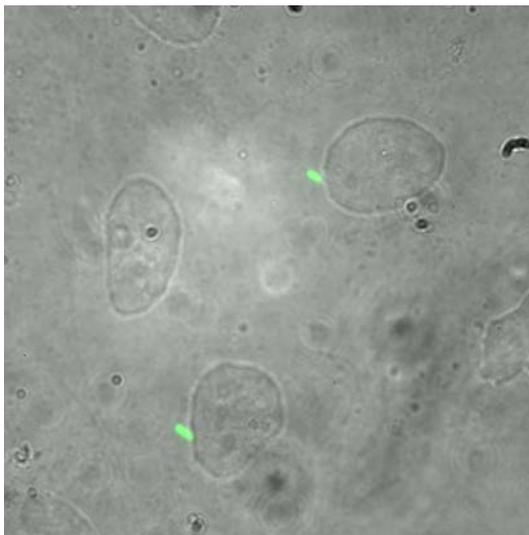
Bindung an Tumorzellen ("Tumorzell Targeting")



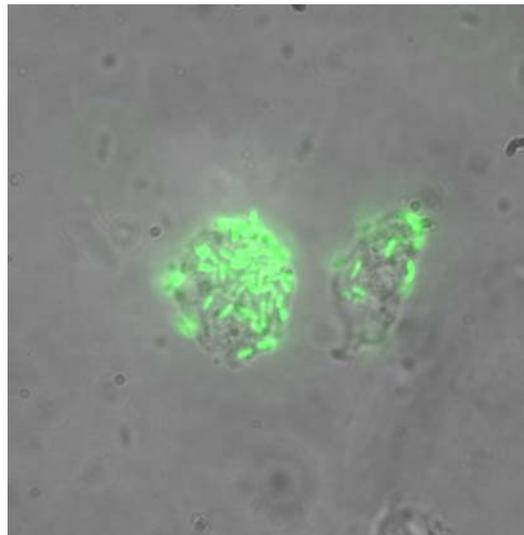
Bindung an Tumorzellen ("Tumorzell Targeting")

Bindung von *E. coli* an Zellen die EGFR überexprimieren (A431)

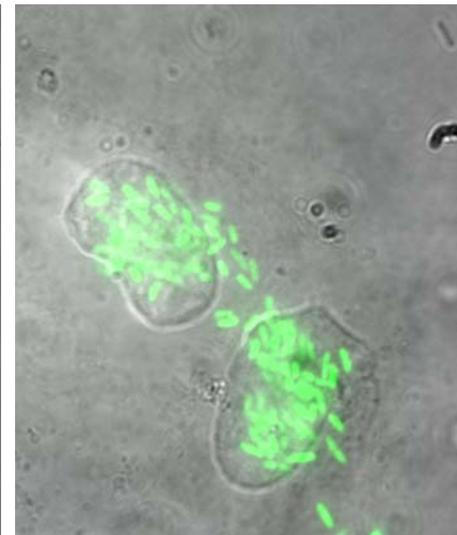
Kontrolle (SDH)



scFv



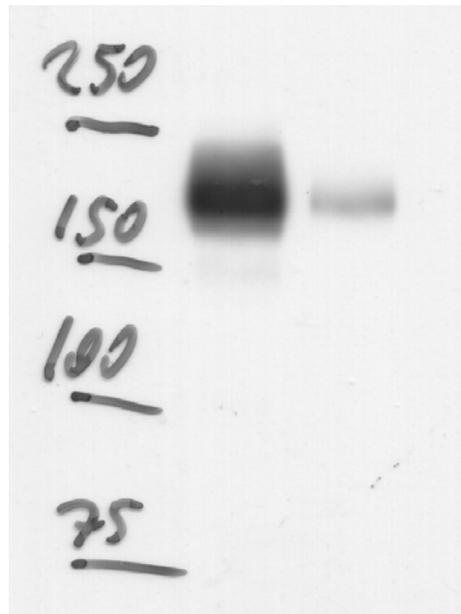
tcFv



Bindung an Tumorzellen ("Tumorzell Targeting")

Silencing des EGFR in A431 Zellen durch siRNA

MW/kDa 1 2

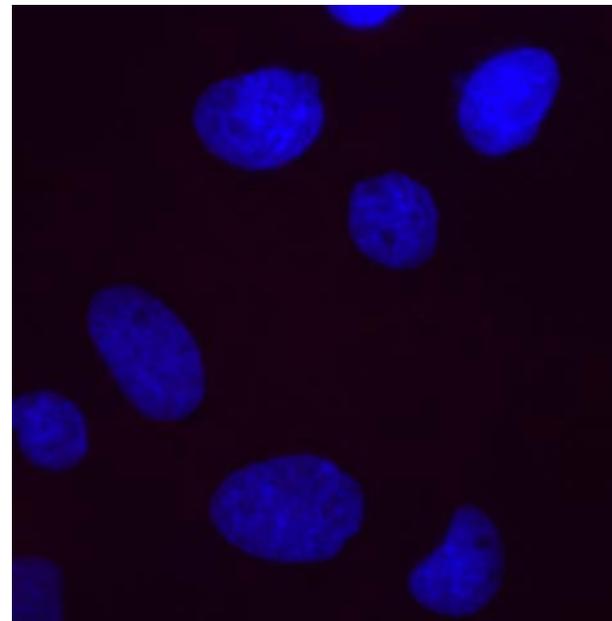
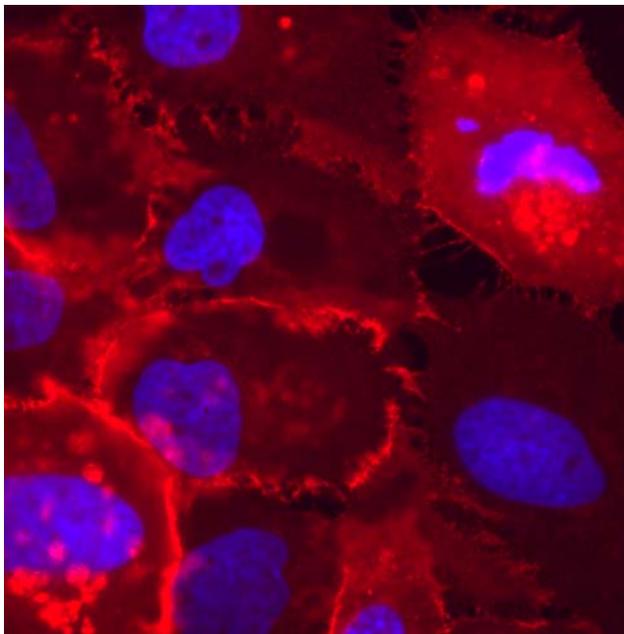


1: Kontroll-siRNA

2: EGFR-siRNA

Bindung an Tumorzellen ("Tumorzell Targeting")

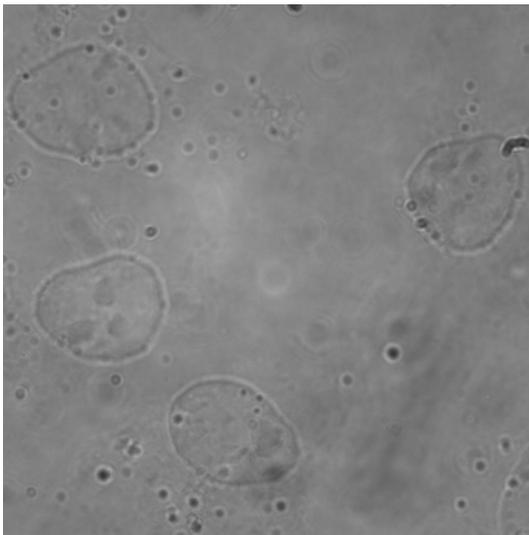
Silencing des EGFR in A431 Zellen durch siRNA



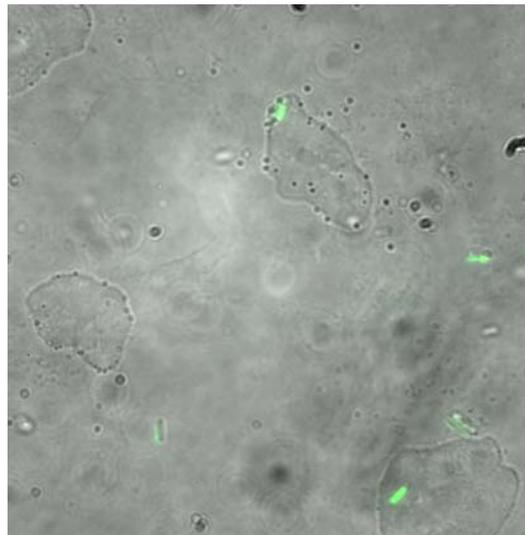
Bindung an Tumorzellen ("Tumorzell targeting")

Silencing des EGFR in A431 Zellen durch siRNA

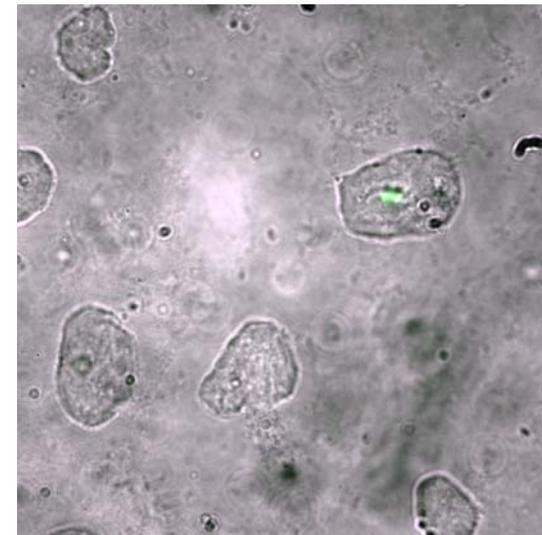
Kontrolle (SDH)



scFv

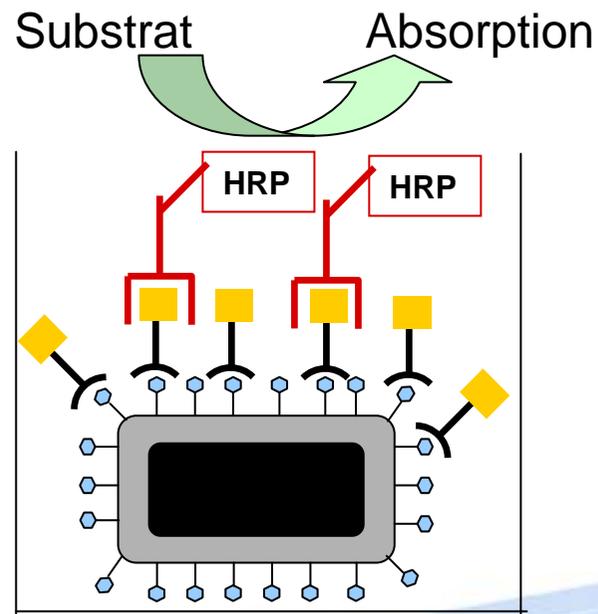


tcFv



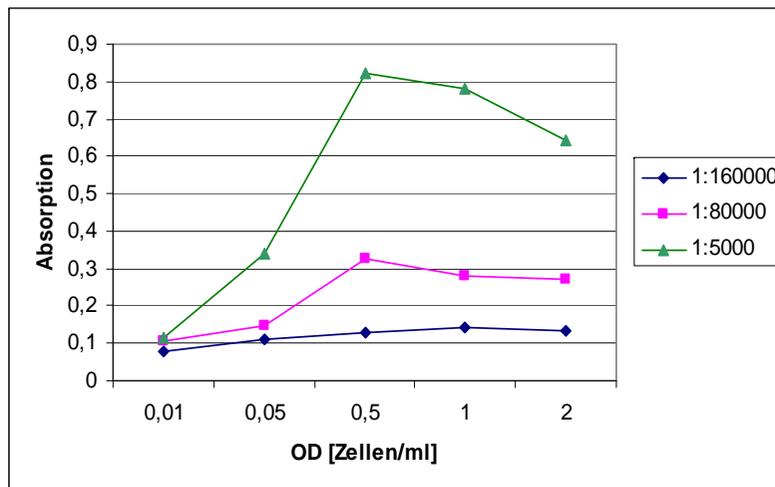
ELISA (enzyme-linked immunosorbent assay)

Basis: *E. coli* Zellen mit einem durch Autodisplay an der Zelloberfläche exprimierten Rheuma-assoziierten Antigens (RAA)

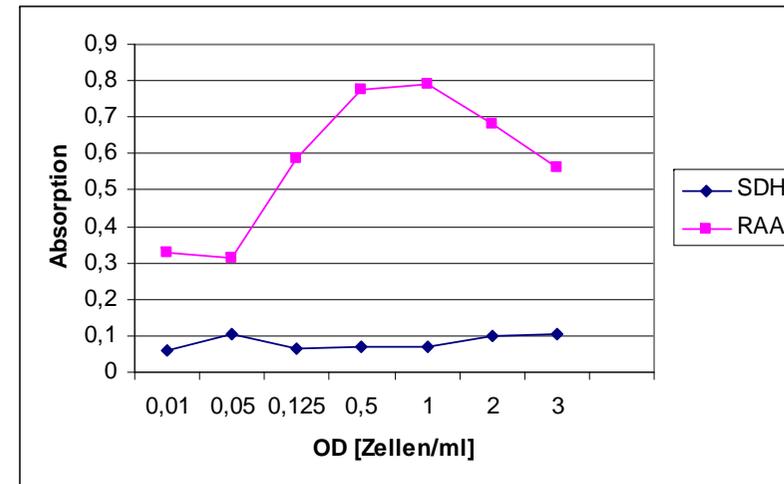


ELISA (enzyme-linked immunosorbent assay)

Konzentrationsreihe des prim. AK

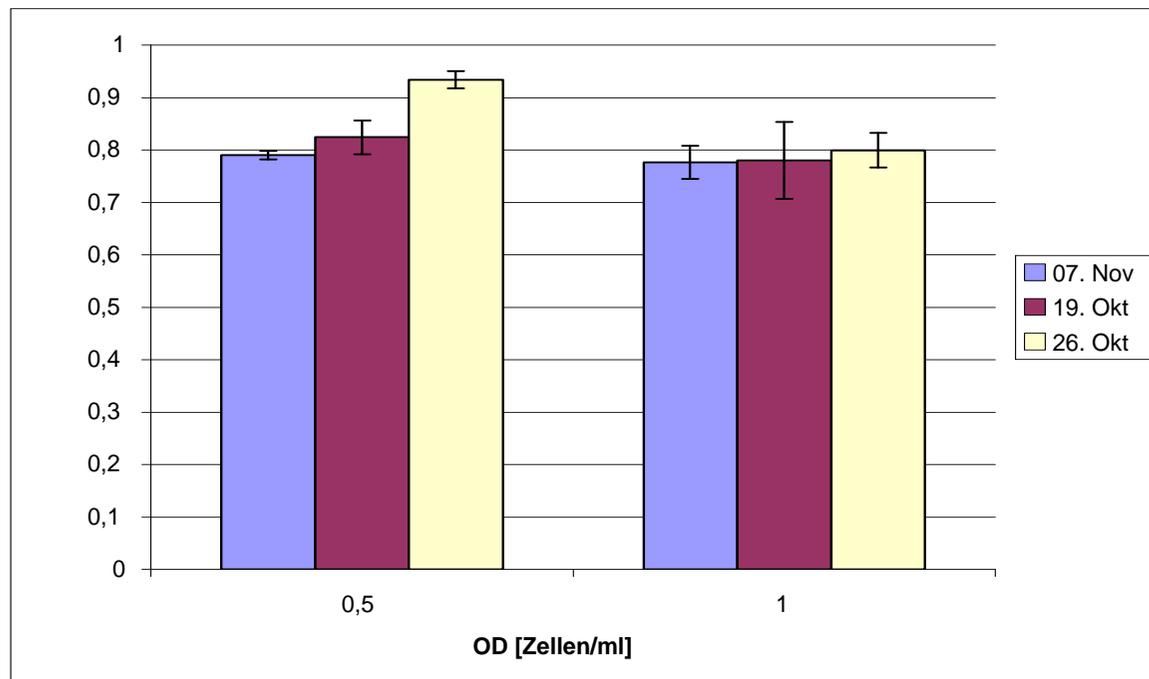


Spezifität des ELISAs



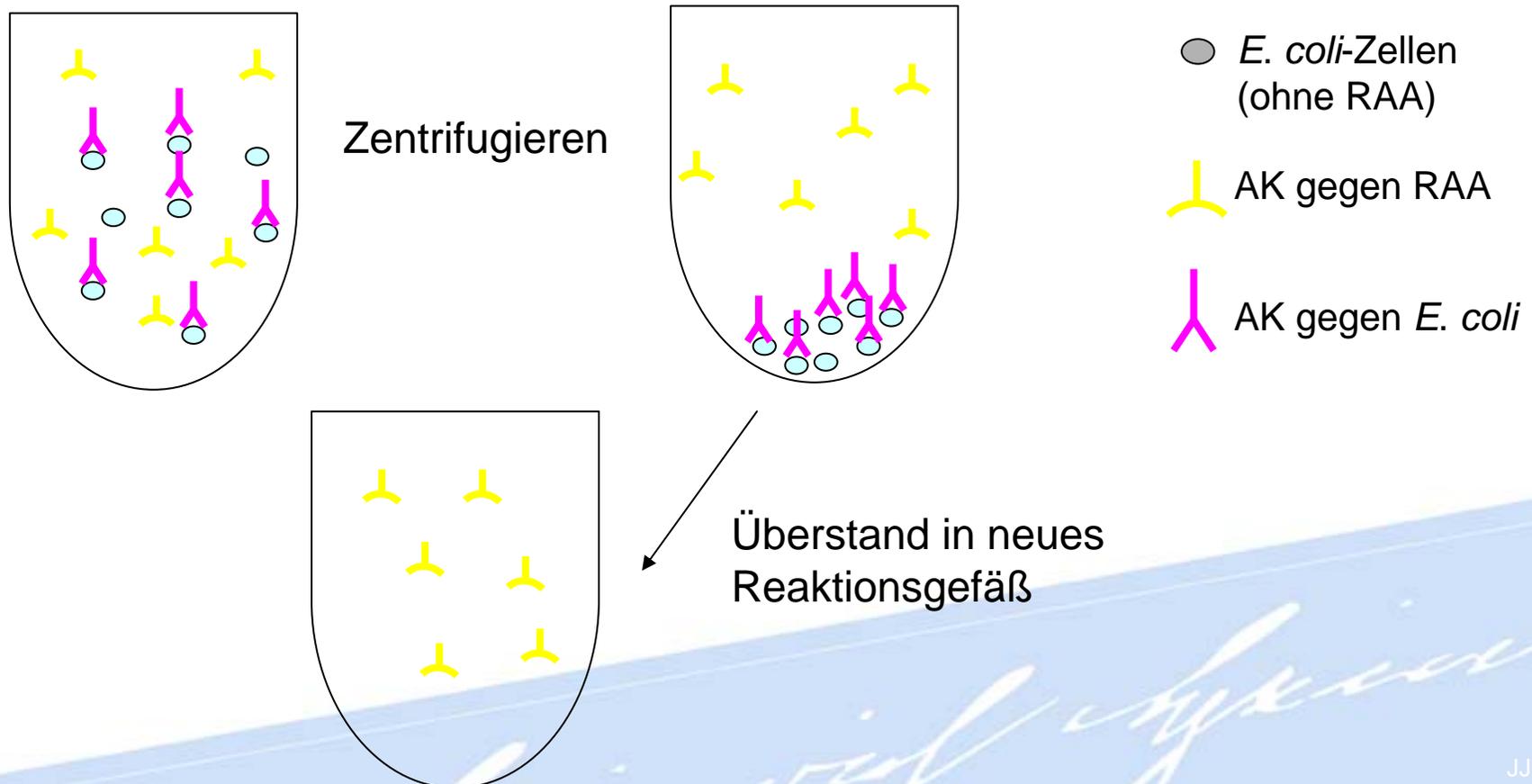
ELISA (enzyme-linked immunosorbent assay)

Reproduzierbarkeit der erhaltenen Werte



ELISA (enzyme-linked immunosorbent assay)

Schematische Darstellung der Preabsorption des
Patientenserums mit *E. coli*-Zellen



Zusammenfassung

Autodisplay –
eine neue Plattformtechnologie
zur Analytik und zur evolutiven Entwicklung
von Biologicals

Surface Display als Werkzeug in der Analytik und der evolutiven Entwicklung von Biologicals

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Jae-Chul Pyun, Lars-Oliver Klotz, Matthias Schneider und Joachim Jose**

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Materials Science and Engineering, Yonsei University, Seoul, Korea,

Molecular Ageing Research, IUF Düsseldorf,

Center for Rheumatology, HHU Düsseldorf.