



**The Abdus Salam
International Centre for Theoretical Physics**



2268-20

**Conference on Nanotechnology for Biological and Biomedical
Applications (Nano-Bio-Med)**

10 - 14 October 2011

Multifunctional Linker Systems for Design of Nano Bio Hybrid Elements

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Multifunctional Linker Systems for Design of Nano Bio Hybrid Elements

Ljiljana Fruk

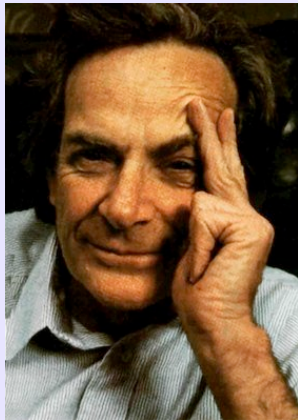
Karlsruhe Institute of Technology, Germany

DFG-Centre for Functional Nanostructures

Bionanotechnology

Use of organic and biomolecular species to design nanodevices and assemblies - at least one component in 1-100 nm scale.

Interdisciplinary nature - combination of life and physical sciences and engineering.



Richard Feynman, 1959
There's Plenty of Room at the Bottom

The Borg -part human, part machine hybrids who assimilated entire races by injecting them with nanoprobes, which altered the host DNA, and changed them into Borg. These nanodevices also served as communicators which gave the consensus a communal awareness.



Borg Queen
Star Trek

Nanoparticles

Type	Examples	Size (nm)
Metals	Au	2-150
	Ag	1-180
	Pt	1-20
	Cu	1-150
Metal oxides	TiO ₂	3-50
	ZnO	1-30
Semiconductors	CdX (X=S,Se,Te)	1-20
Quantum Dots	ZnX (X=S,Se,Te)	1-20
Magnetic	Fe ₃ O ₄	6-40
Polymer	Various - PEG, Chitosan...	50-1000

Noble metals

- high surface plasmons, large extinctions, catalytic properties (recently thermal for Au)

Semiconductors

- excellent fluorescent properties - biolabelling

Magnetosomes

- magnetic labelling

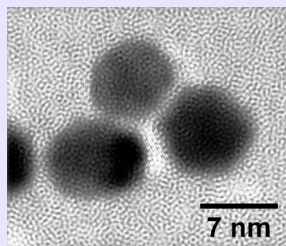
Oxide nanoparticles

TiO₂ - photosensitivity, reactive oxygen species generation

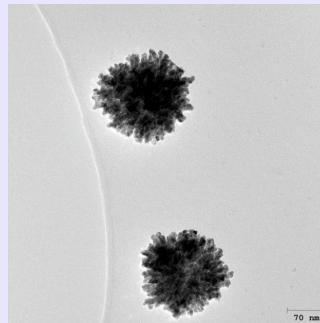
Nanoparticle Preparation

Polyol method*

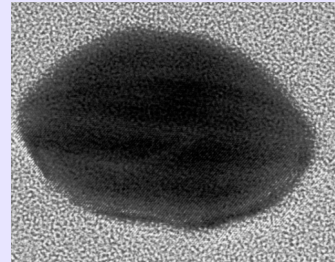
- metal precursor, surface polymer (polyvinyl pyrrolidone) - control over the NP shape
- heating in ethylene glycol - solvent/reducing agent



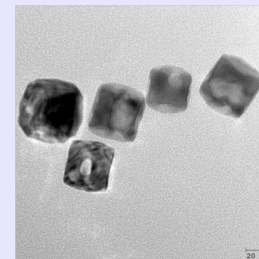
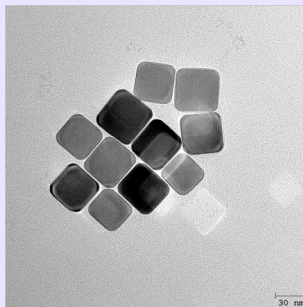
Pt



Au



Au



Ruoli Wang, Cheng Chen

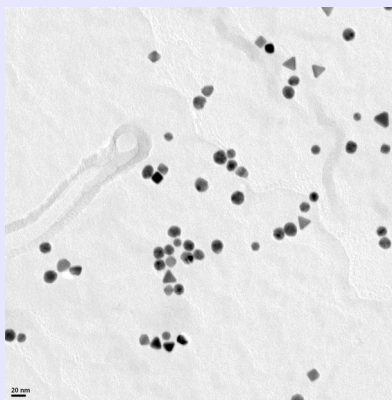
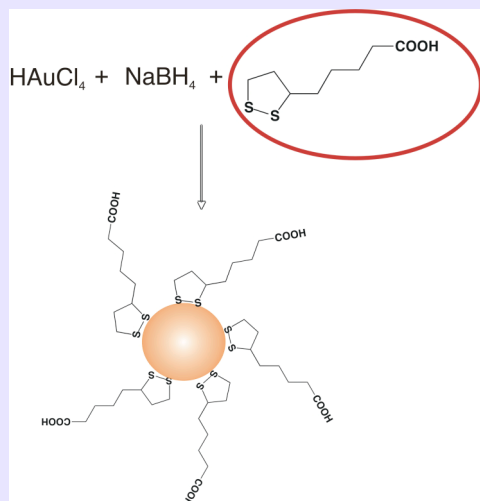
Y.Sun, Y.Xia, *Science* **2002**, 298, 2176.

R.Wang et al., submitted.

J. Chen et al, *Small* 2010, 6, 811

NP Preparation - Metal NP

One pot synthesis - use of reducing agents, stabilising ligands with suitable anchoring group

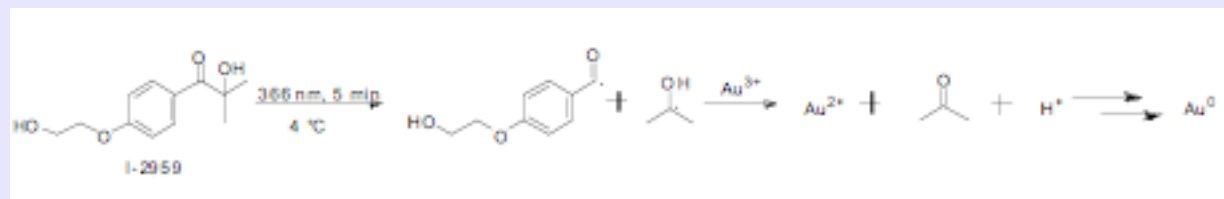


Both Au and Pt NP

Dania Kendziora

M. Altemöller

Photosensitive reducing agent - for mild reduction - Irgacure 2959*



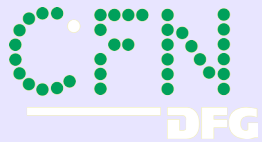
L. Maretti et al, J. Am. Chem. Soc. 2009, 131, 13972

Bioimaging/Diagnosis/drug delivery

- trackable, fluorescent particles
- design of biofriendly, fluorescent linkers
- NP anchoring group
- water soluble linker
- biofriendly chemistry - click, amide coupling, Michael reaction

Metal/metal oxide NP - inherently non fluorescent -
biofunctionalisation - depending on the system

Linkers - interface between inorganic or polymer cores
and biomolecules

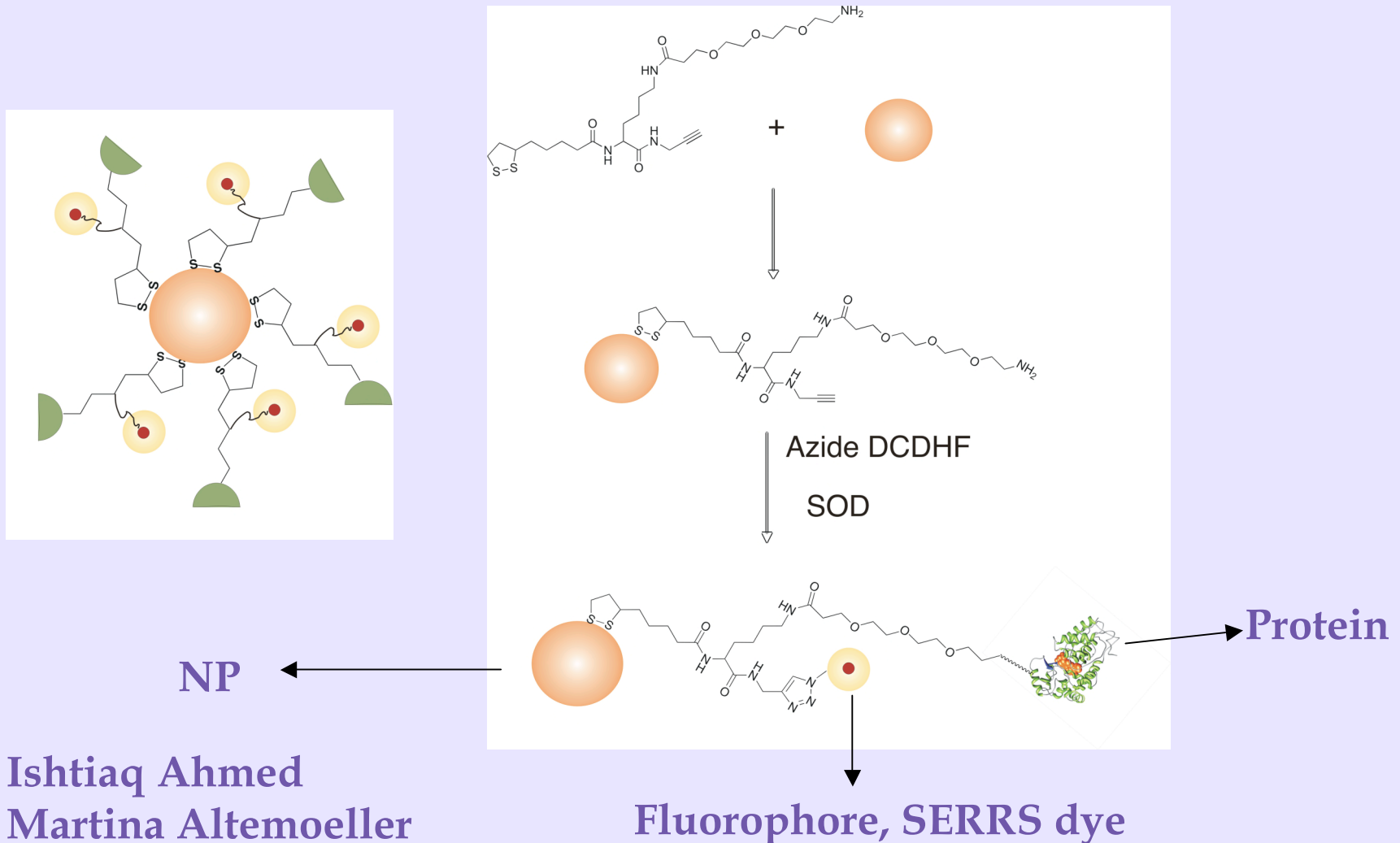


Chemically Synthesized Linkers

1. Define what you want to do with it
 - choice of proper functional group
2. Try to find **suitable and affordable chemistry** to make it
3. Find **suitable NP/surface anchoring group**
ie. thiols for Au, catechols for oxides,
4. Think about the **visualisation/characterisation method**

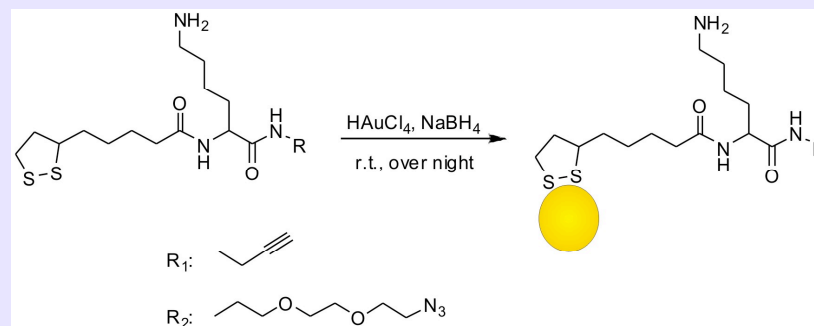
Linkers - NP/Biomolecule Interface

Visualisation - both for drug delivery and imaging

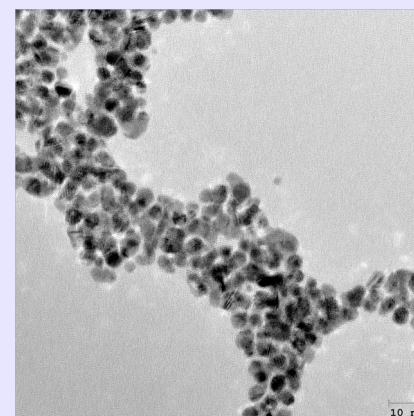
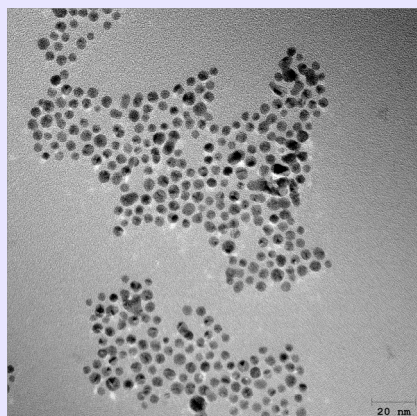
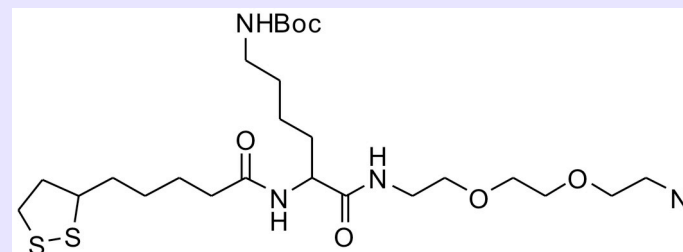
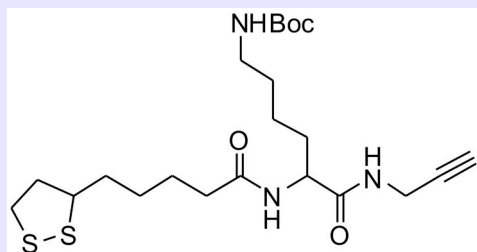


Ishtiaq Ahmed
Martina Altemoeller

Trifunctional Linkers



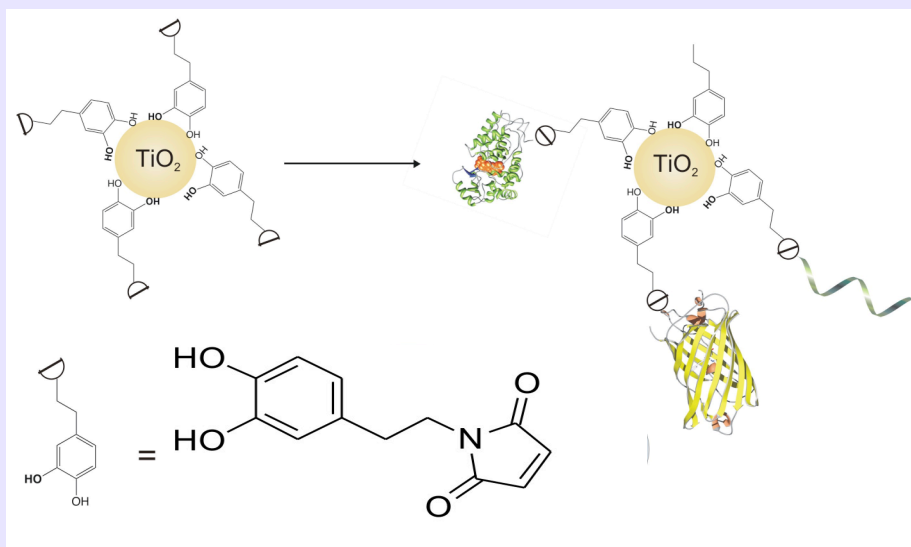
Cu catalysed click chemistry



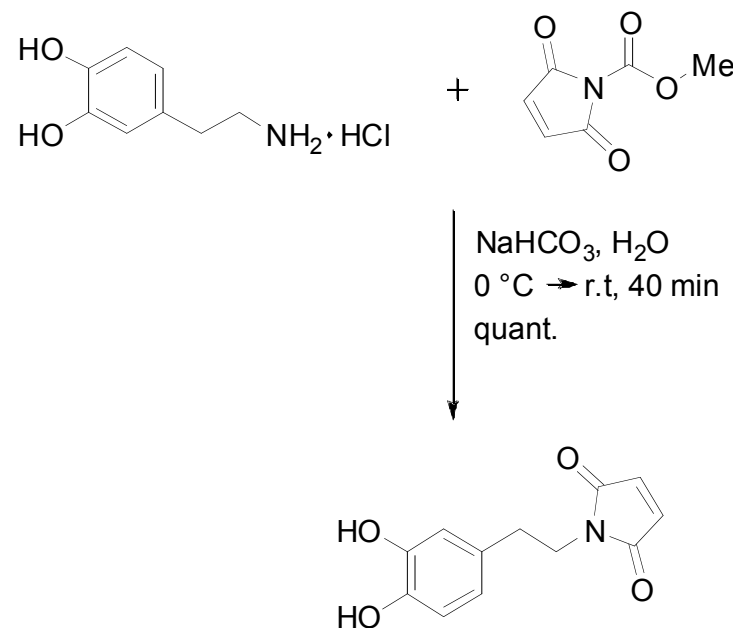
Au NPs
Dania Kendziora

Modification of TiO₂ NPs

Catechol linkers or any oxygen rich species



Dopamine Maleimide

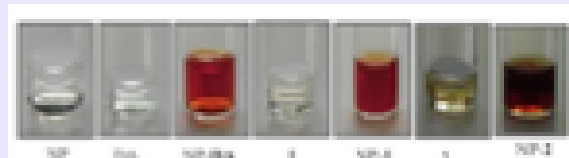


Bianca Geiseler

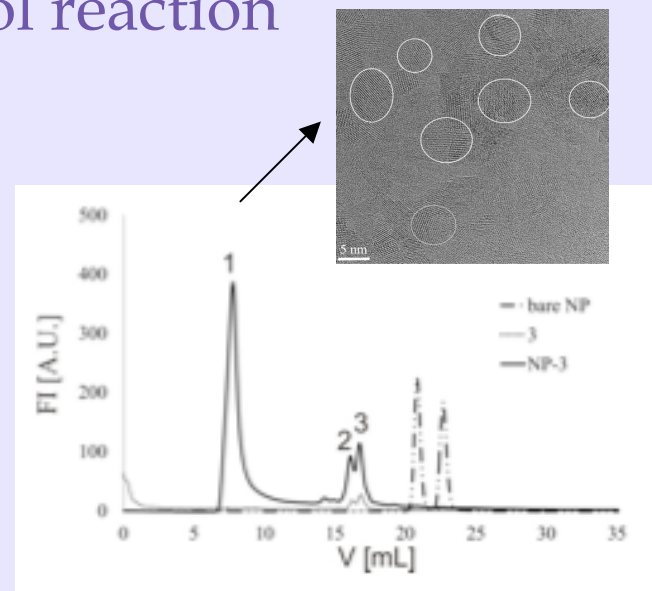
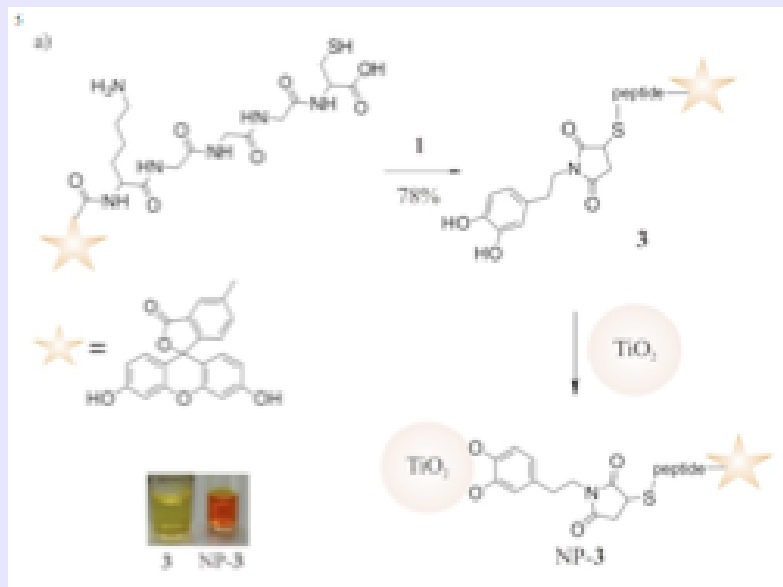
B. Geiseler, L. Fruk, J. Mat. Chem. 2011, in press

Modification of TiO₂ NPs

Charge transfer complex formation - absorbing in visible

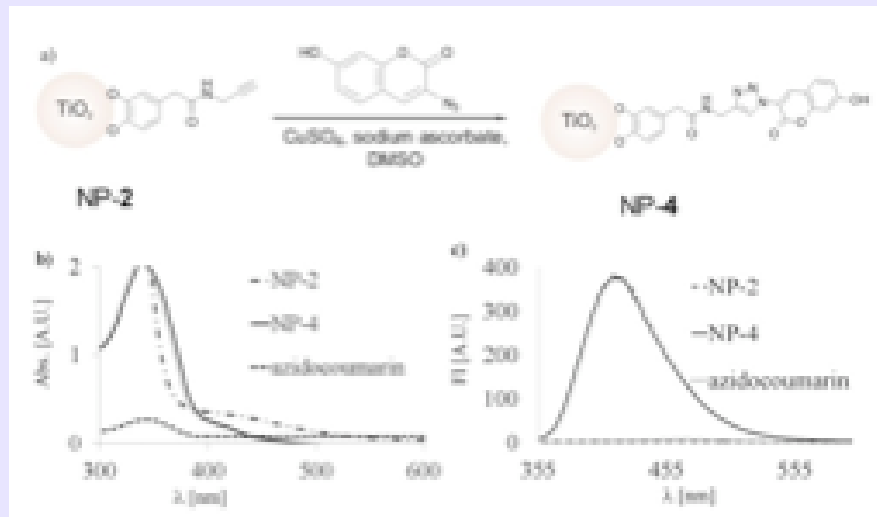


Peptide addition - maleimide thiol reaction



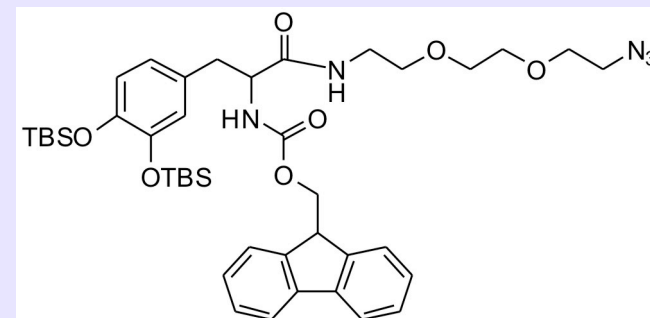
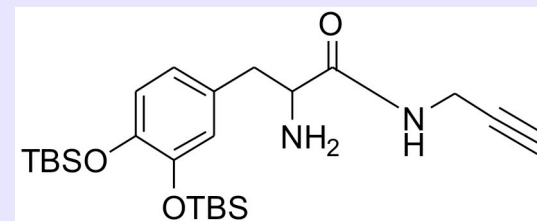
Size exclusion chromatography

Modification of TiO₂ NPs



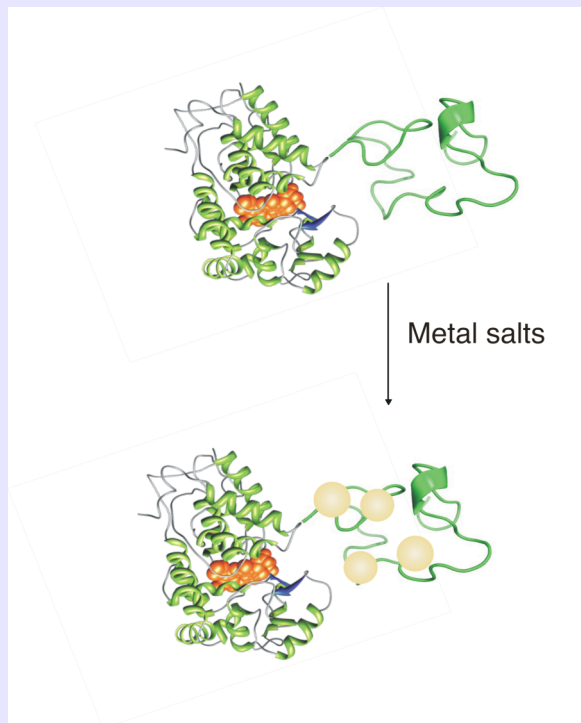
Ishtiaq Ahmed
Bianca Geiseler

Click chemistry
on TiO₂ surface



Peptide Based Linkers

Metallothioneins (MT) - cystein rich peptides, detoxification, cancer resistance - fusion proteins for TEM imaging
(C.P.Mercogliano et al., *J.Str..Biol.* 2007, 160, 70.)



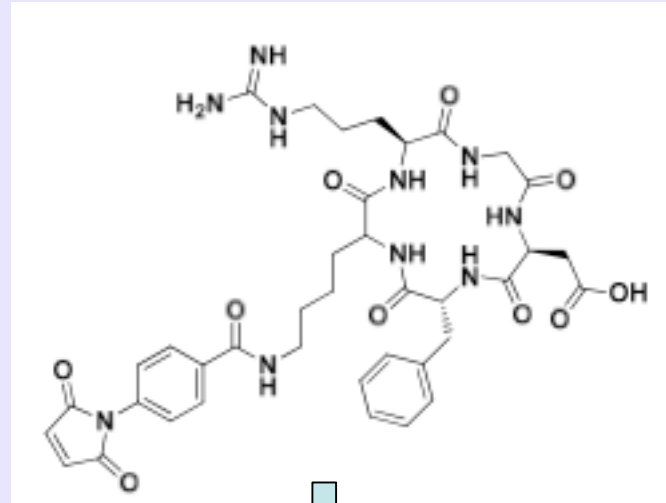
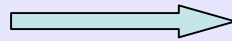
**Fusion protein genetically engineered
MT-maltose binding protein**
- growth of NP with the help of photo
Activable reducing agent

D. Kendziora

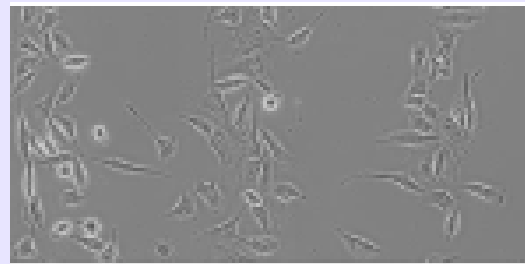
D. Kendziora et al, submitted

Bifunctional Peptide linker

RGD cyclic peptide for cell attachment

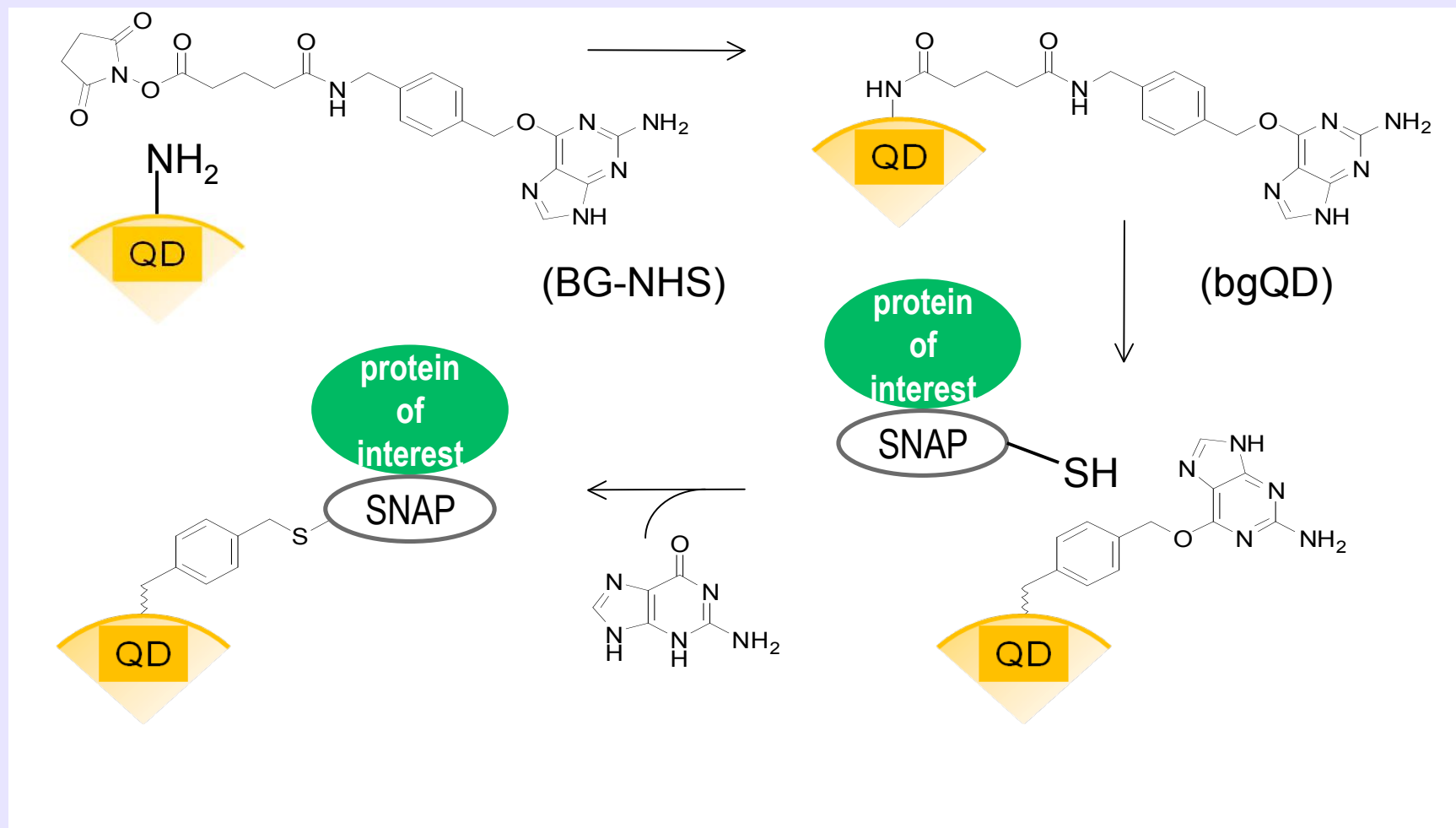


RGD
-Specific
Integrin
binding



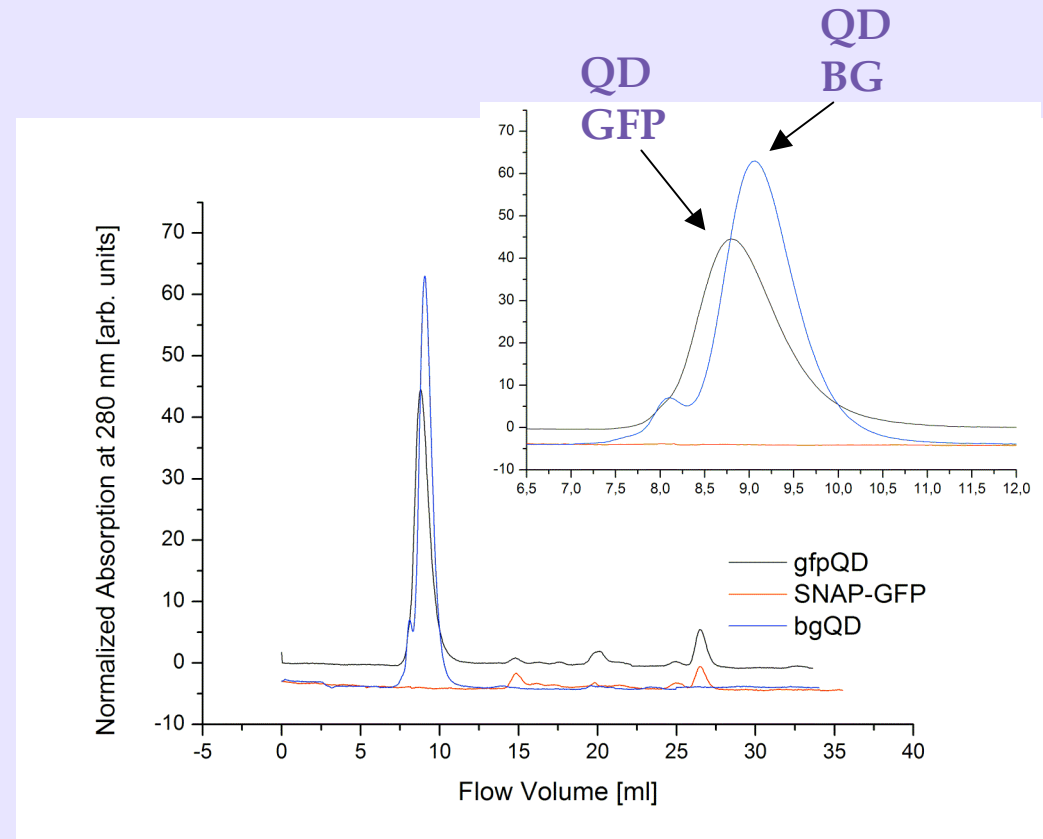
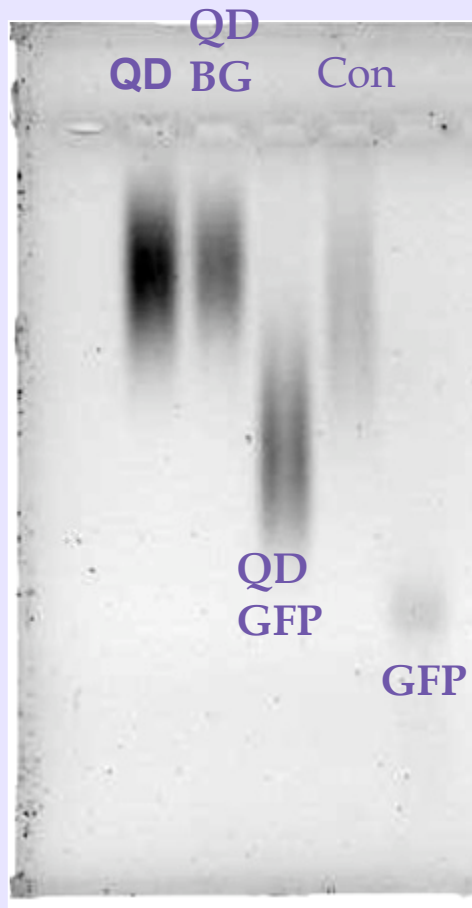
Lukas Stolzer,

Protein Tag Based Linkers



Protein Tag Based Linkers

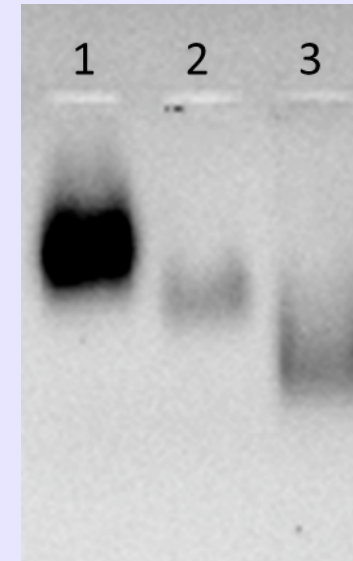
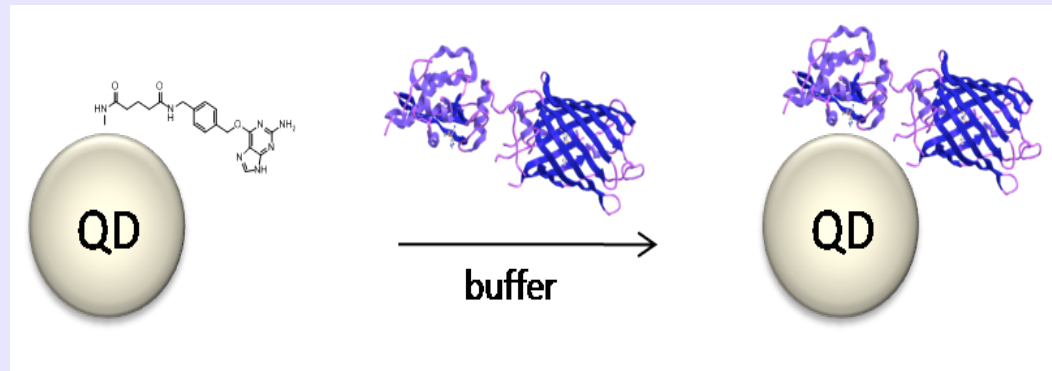
Quantum Dot Modification



Size exclusion chromatography

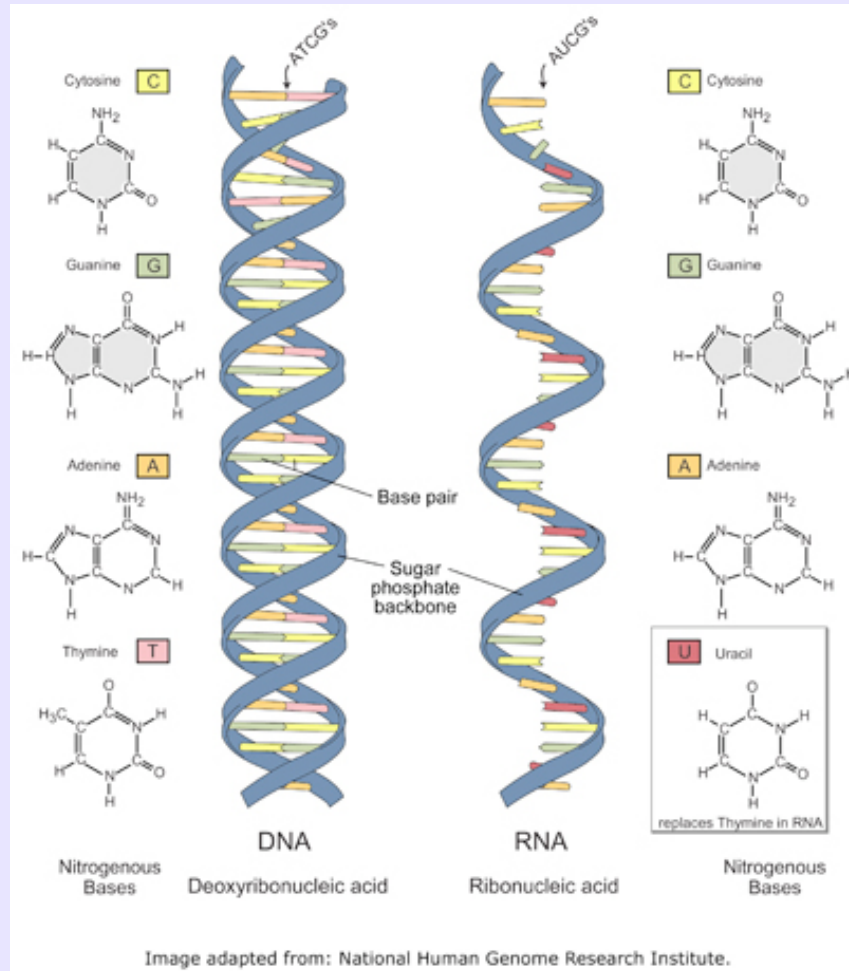
Protein Tag Based Linkers

E-cadherine - cell-cell contacts, tissue growth and integrity



Next step: interaction and cascade activation of E-cadherine

DNA as a functional linker

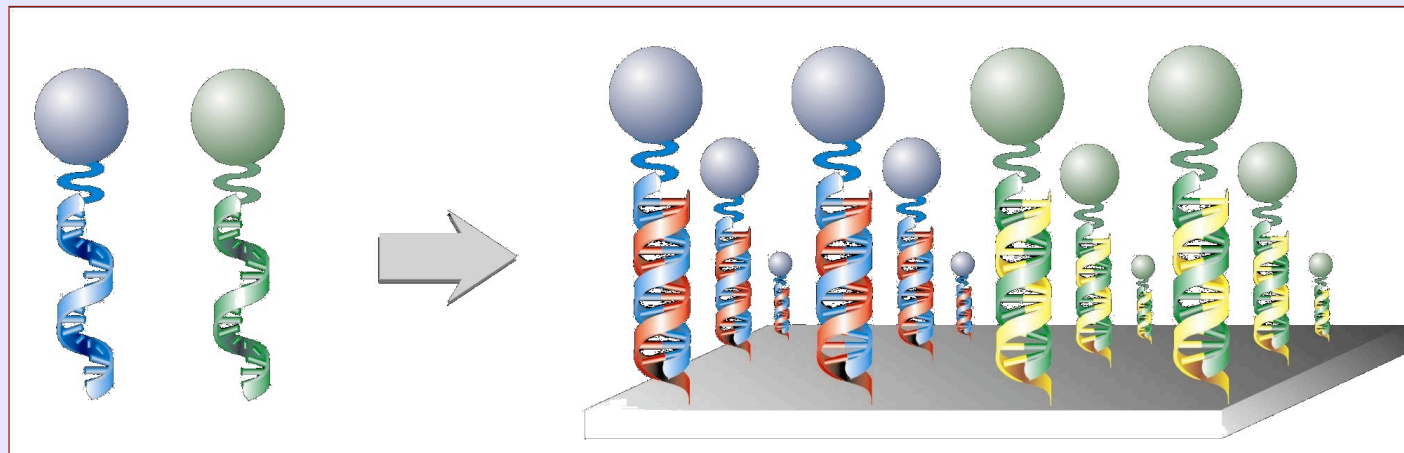


Watson Crick
Base Pairing

DNA as a Functional Linker

DNA-Directed Immobilisation (DDI)

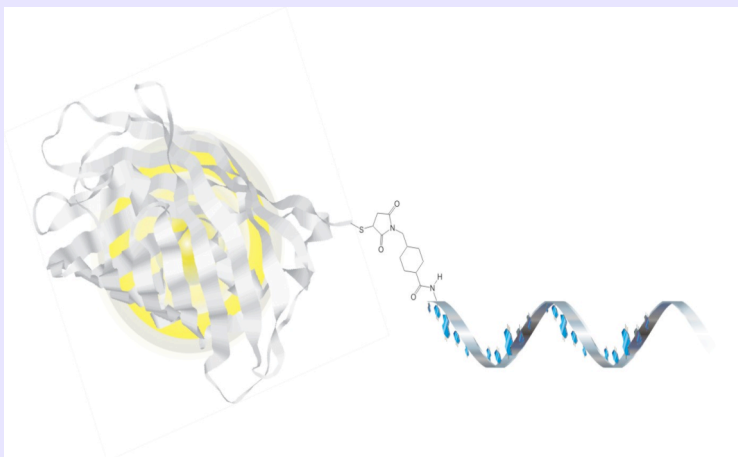
- Chemically mild, reversible immobilisation of molecules and inorganic particles
- Based on specificity of DNA base-pairing
- Can be used with various solid surfaces



DNA as a Functional Linker

DNA can be synthesized and modified - robust molecule
It can be attached to small molecules, peptides and proteins.

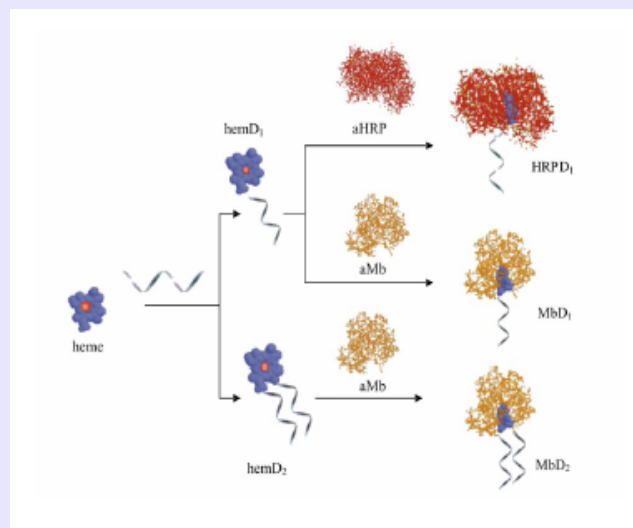
EYFP-DNA conjugate



F.Kukolka, C.M. Niemeyer,

Org. Biomol. Chem., 2004, 2, 2203

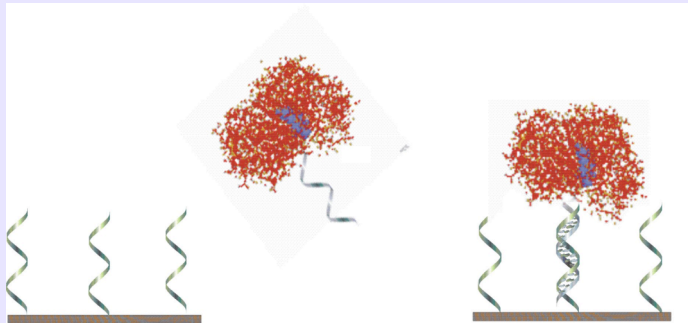
Heme Enzymes



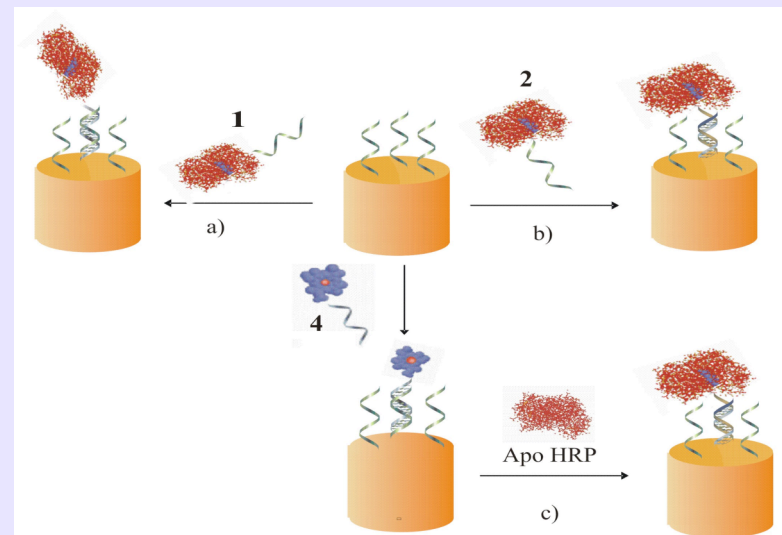
Lj.Fruk, C.M. Niemeyer,
Angew.Chem.Int.Ed.,

2005, 335, 943-948

Attachment to solid surfaces

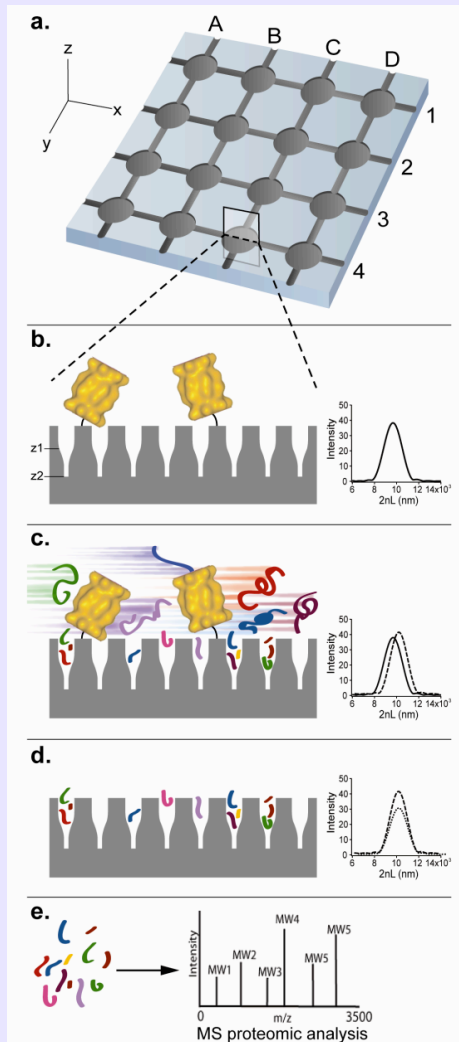


Electrochemical Sensor

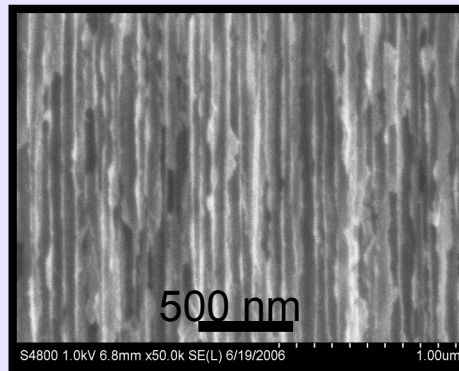


DNA as a Functional Linker

Protease Biosensor



Porous silica



Photoluminescent

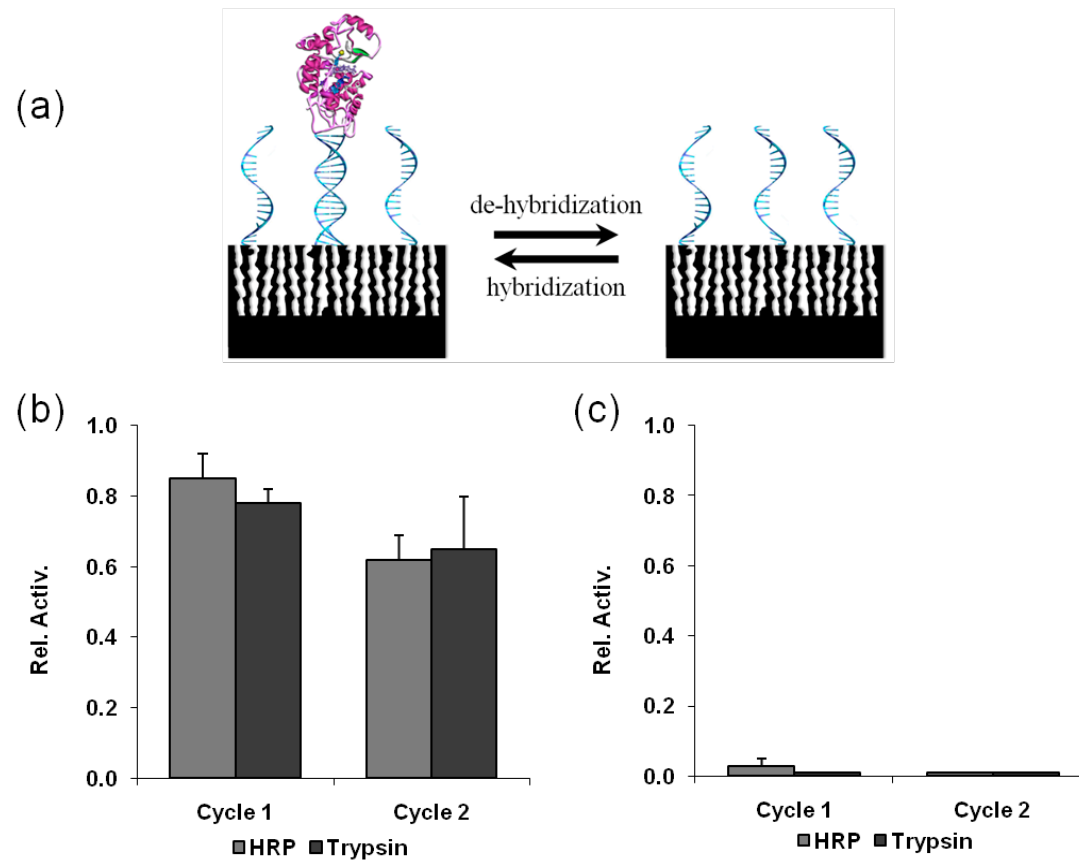


Capture of the small fragments
in the pores
- MS analysis

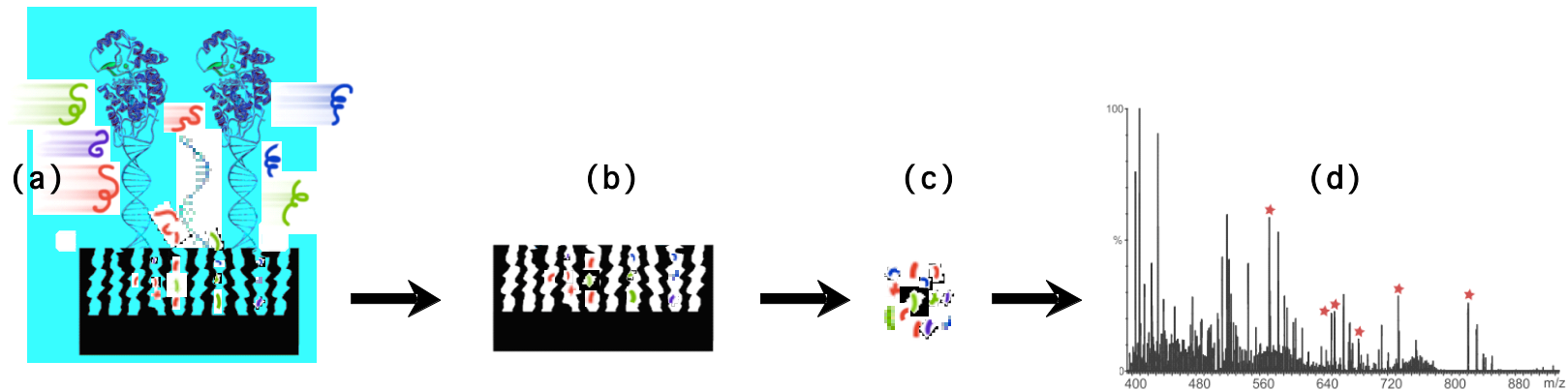
Giorgi Shtenberg
Sinem Engin

G. Shtenberg et al, submitted.

Protease Biosensor



Protease Biosensor



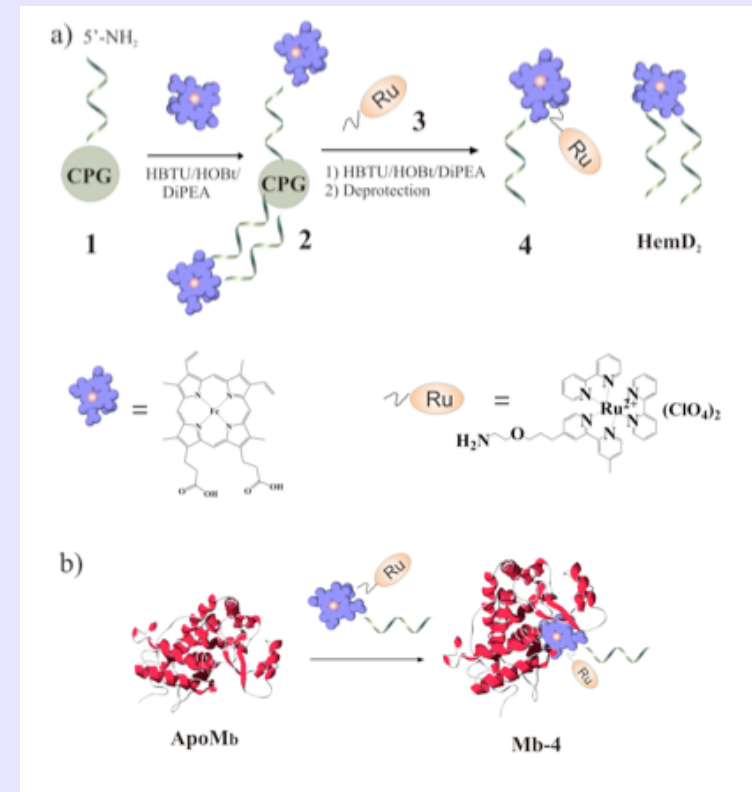
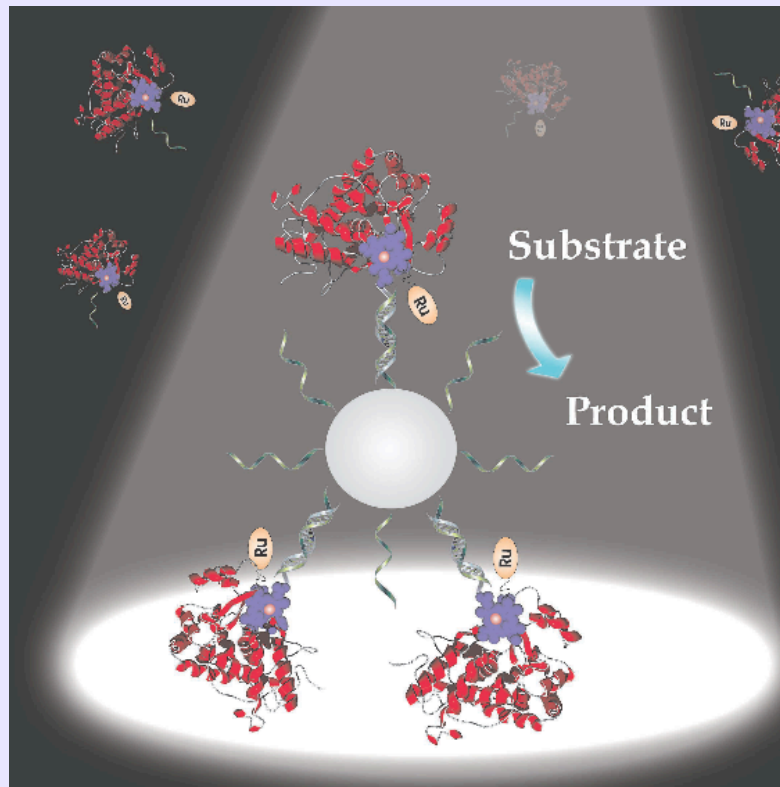
(e)

1	MKWVTFISLL	LLFSSAYSRG	VFRDRTHKSE	IAHRFKDLGE	EHFKGLVLIA
51	FSQYLQCCPF	DEHVKLVNEL	TEFAKTCVAD	ESHAGCEKSL	HTLFGDELCK
101	VASLRETYGD	MADCCCKQEP	ERNECFLSHK	DDSPDLPKLK	PDPTLDCDEF
151	KADEKKFWGK	YLYEIARRHP	YFYAPELLEY	ANKYNGVFQE	CCQAEDKGAC
201	LLPKIETMRE	KVLASSARQR	LRCASIQKFG	ERALKAWSVA	RLSQKFPKAE
251	FVEVTKLVTD	LTKVHKECCH	GDLLECADDR	ADLAKYICDN	QDTISSKLKE
301	CCDKPLLEKS	HCIAEVEK DA	IPENLPPLTA	DFAEK _DVCK	NYQEAKDAFL
351	GSFLYEYSR R	HPEYAVSVLL	R LAKEYEATL	EECCAKDDPH	ACYSTVPDKL
401	KHLVDEPQNL	IKQNCQFEK	LGEYGFQNAL	IVR YTR KVPQ	VSTPTLVEVS
451	R SLGKVGTRC	CTKPESERMP	CTEDYLSLIL	NRLCVLHEKT	PVSEKVTKCC
501	TESLVNRRPC	FSALTPDETY	VPKAFDEKLF	TFHADICTLP	DTEKQIK KQT
551	ALVELLK HKP	KATEEQLK TV	MENFVAFVDK	CCAADDKEAC	FAVEGPKLVV
601	STQTALA				

Trypsin digestion investigated - specific fragments found

DNA as a Functional Linker

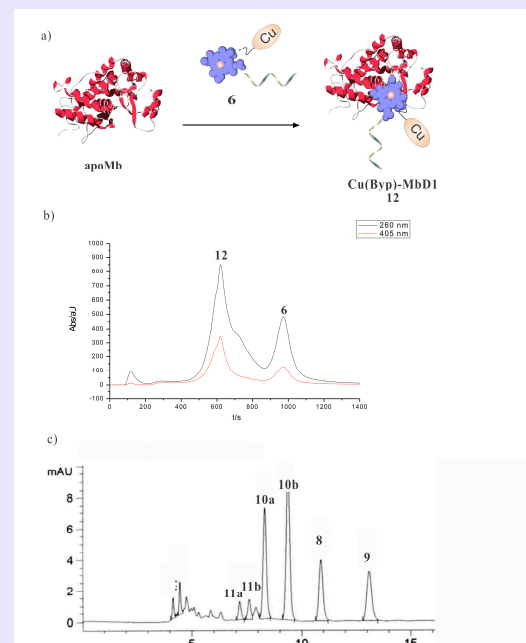
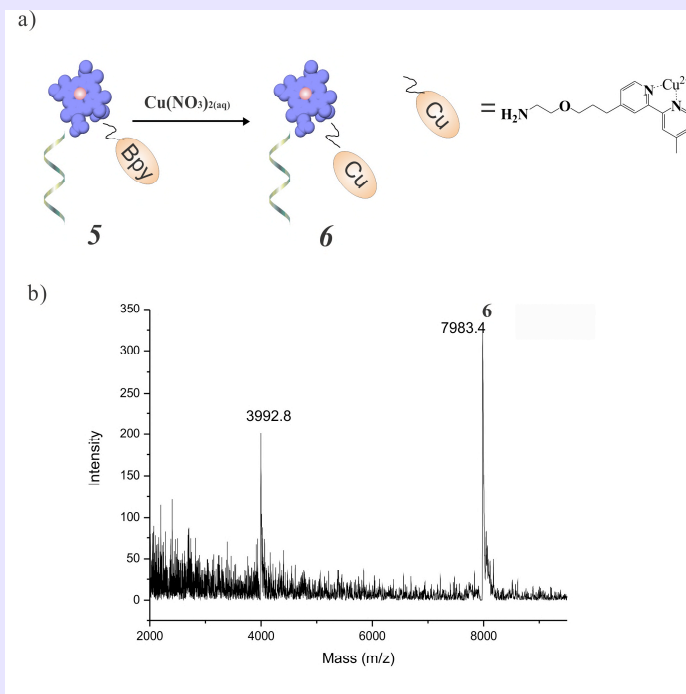
Photoactivable enzymes - addition of light sensitive group to cofactor



Immobilisation on magnetic beads for separation

*C.H.Kuo *et al.*, *Chemistry - An Asian J.* 2009, 4(7),1064.

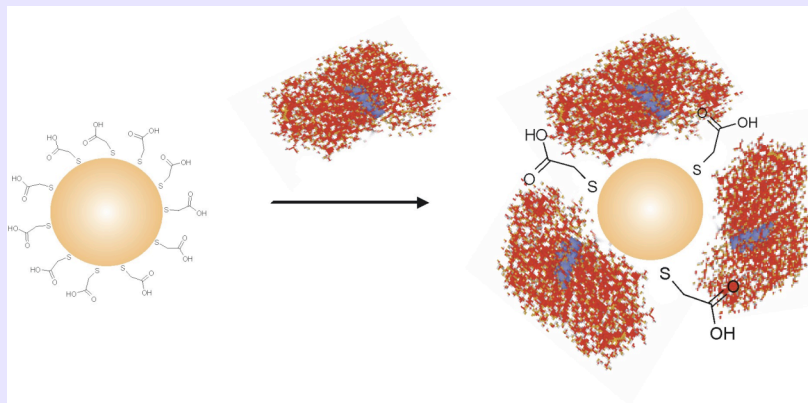
Cu complex + DNA for enantioselective reactions



Artificial heme replacing natural in myoglobin
 - artificial myoglobin enantioselective catalyst

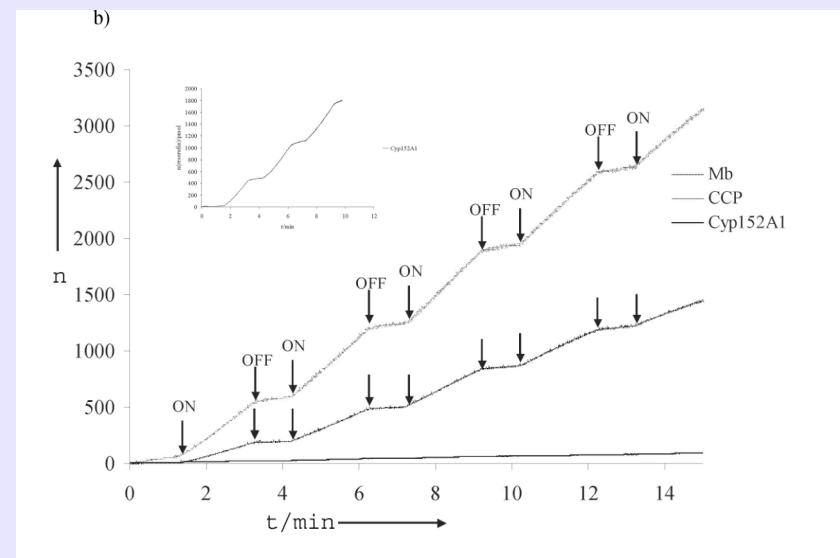
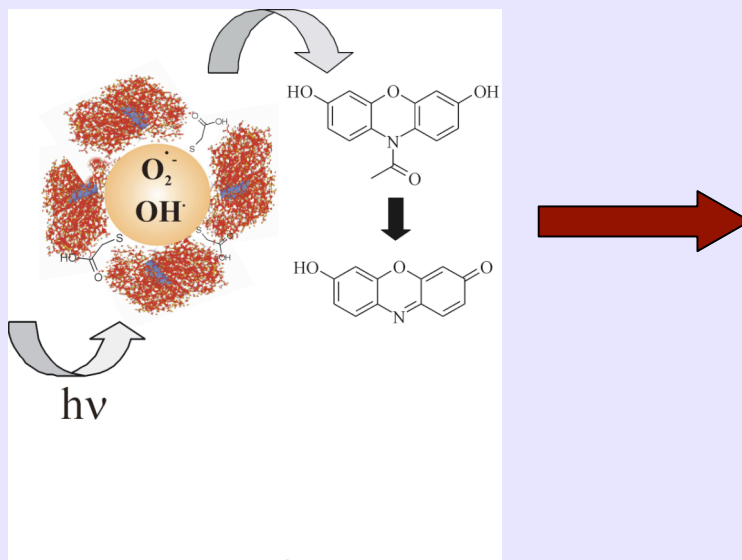
No Linker?

Photocatalysts - electrostatic attachment onto CdS QDs



3- 4 HRP enzymes per QD

Slightly positively charged enzyme - negatively charged QD's



Conclusions

- Careful design of linkers important
- adjusted to the type of application
 - biorthogonal?
 - reversible attachment?
 - detectable?
 - water soluble?
 - stabilising?
 - easy to make?

When God created bulk, surfaces were left to the Devil
W. Pauli

Acknowledgements



Group

Bianca Geiseler
Ishtiaq Ahmed
Dania Kendziora
Cheng Chen
Ruoli Wang

Lukas Stolzer
Sinem Engin
Martina Altemoeller
Philipp Mueller



Collaborators

Ester Segal, Giorgi Shtenberg, Technion
Michal Sharon, Weizmann Institute
Daniel Schaadt, KIT

Yu Chueh Chua, Tsing Hua University, Taiwan



Funding:

DFG Excellence Initiative, EU, DAAD

Optical properties of PSi

Fabry-Pérot Interference

$$m\lambda = 2nL$$

Optical thickness

λ - vacuum wavelength

m - integer

n - average refractive index

L - thickness of the porous Si layer

