„ Mechanisms of non-viral gene delivery “

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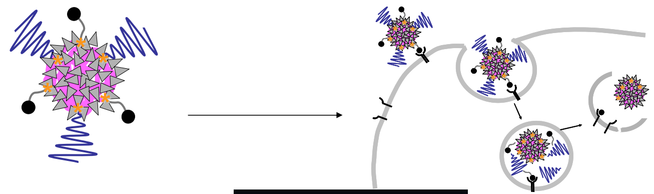
14 06 2008

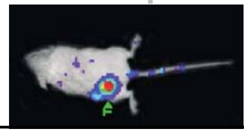
Programmed Nanosystems – ‘Synthetic Viruses’

- Specific delivery and effective drug release
- Timely operation of multiple tasks

‚Smart‘ Nanosystems **Extra & Intra - Cellular Pathways**

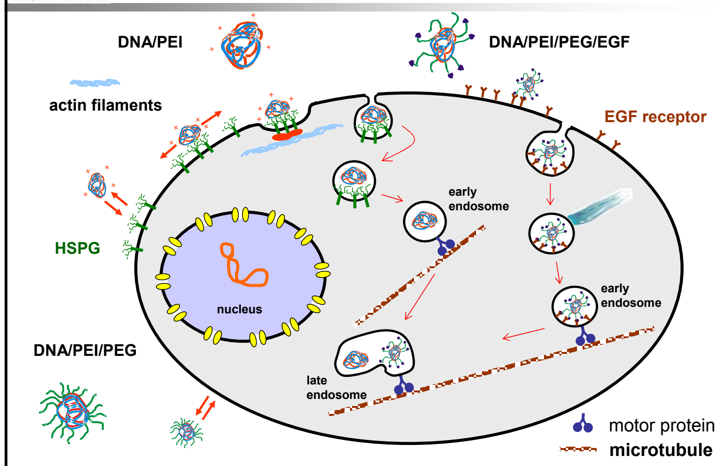


Shielding function (blood circulation)
Targeting function
Triggered release (at target site/cell)



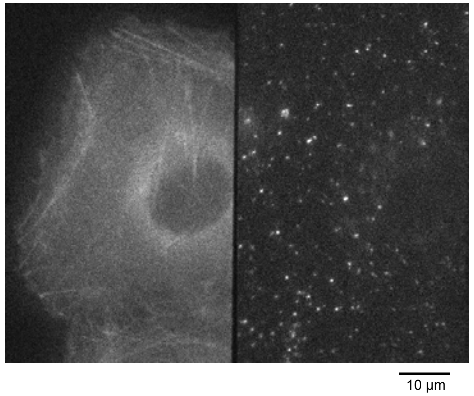
EGF receptor targeted delivery

Interactions with Cells



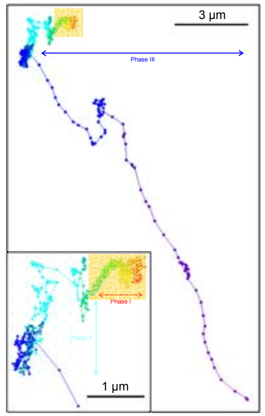
Observation of Single Polyplexes on Living Cells

Cell with actin-GFP labeled cytoskeleton | Cy5-labeled polyplexes

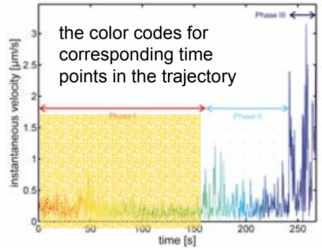


Christoph Bräuchle
Karla de Bruin
Nadia Ruthardt

EGF Polyplex Tracking: Three Phases



tracked for 4:30 min at a frame rate of 300 ms

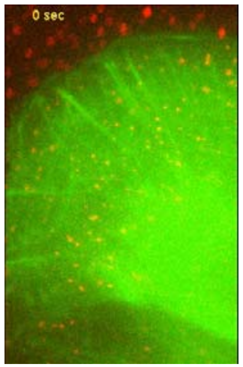


three phases observed:

phase I: slow directed motion

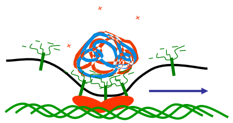
phase II: diffusion

phase III: directed motion along microtubules

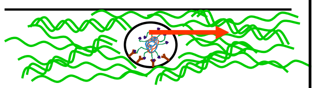


HUH-7 cells / GFP-tagged actin

Phase I
PEI particles on **actin-GFP** cell

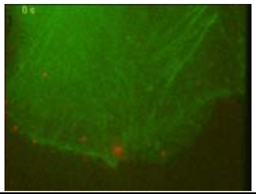


recorded at 5 second intervals for 54 min, 500 ms acquisition time, 75x time lapse



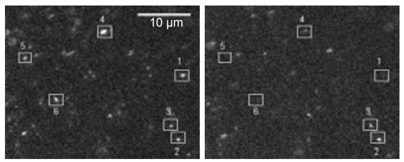
Phase II
(occasionally actin comets)

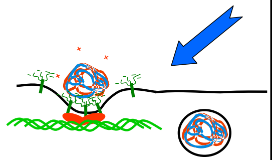
60 x time lapse

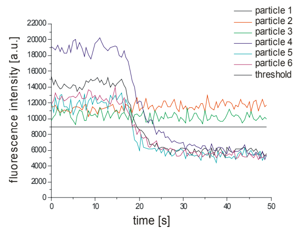


K. deBruin et al, *Mol. Ther. 2007*

Particle Internalization: Quenching Assay





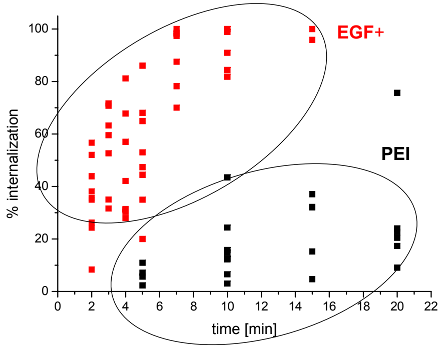


Quenching of Cy3 fluorescence with the membrane-impermeable dye trypan blue

→ only extracellular particles will be quenched

Particle Internalization

Internalization rate of EGF+ and PEI polyplexes in HuH7 hepatoma cells



One data point corresponds to one single-cell measurement

EGF+:

50% internalization within 5 min

PEI:

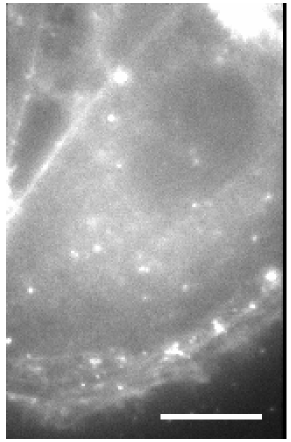
20% internalization within 20 min

K. deBruin et al, *Mol. Ther. 2007*

Active Intracellular Particle Transport

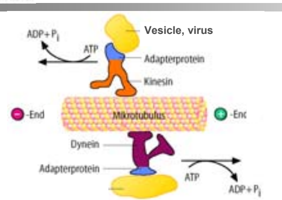
- **PEI polyplex motion NOT linked with actin**

HUH-7 cells / GFP-tagged actin



K v Gersdorff
+ C. Bräuchle lab *Angew. Chem.2006*

Active Intracellular Particle Transport

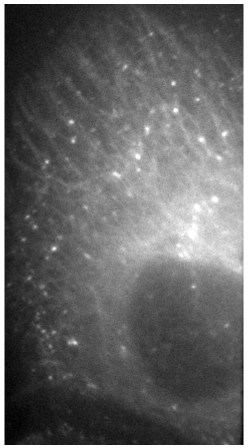


- **Polyplex motion linked with microtubules**
- **Particles within vesicles**

(colocalize with fluid phase marker)

➔endosomal escape is a bottleneck

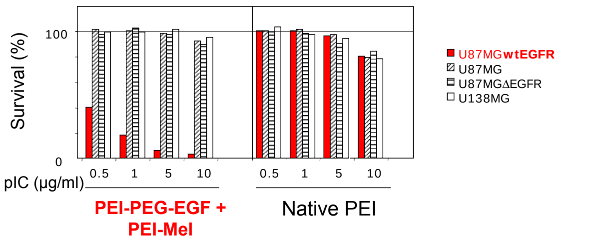
HUH-7 cells / GFP-tagged tubulin



K v Gersdorff
+ C. Bräuchle lab *Angew. Chem.2006*

EGF receptor targeted tumor therapy

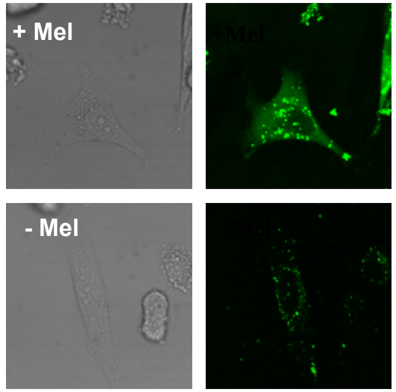
EGFR-targeted dsRNA Poly Inosine/Cytosine Eliminates Glioblastoma Cells



- Intracellular (poly IC) activates antiviral cell mechanisms (apoptosis, cytokines)
- Selectively kills EGFR overexpressing cells within 1 hour !
- EGF receptor targeting plus endosomolytic Mel peptide required

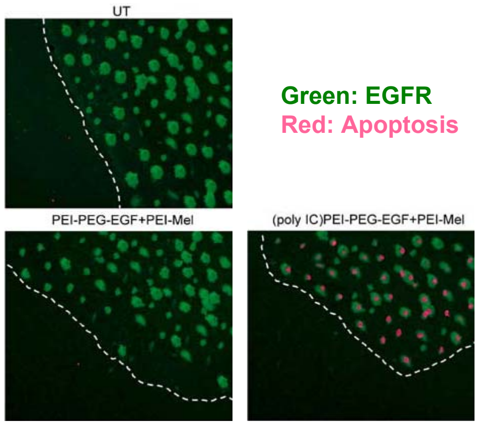
W. Rödl, M. Ogris / **Collaboration with Alexei Shir, Alexander Levitzki** *PLOS Medicine* 2006

EGFR-targeted Poly IC: Intracellular Release by Melittin



Fluorescein - labeled poly IC

EGFR-targeted Poly Inosine/Cytosine Eliminates Glioblastoma Tumors



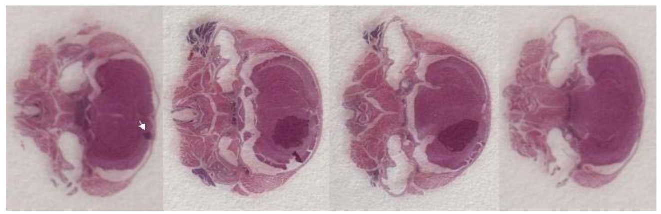
Green: EGFR
Red: Apoptosis

Tumor

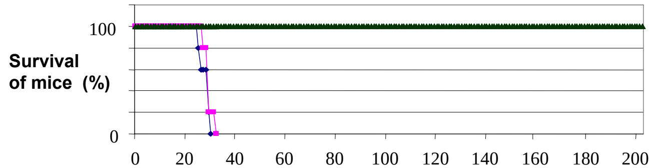
Brain tissue

EGFR-targeted Poly Inosine/Cytosine Cures Mice from Glioblastoma

10 days tumors **20 days tumors**



No treatment **No treatment** **conjugate control** **pIC/PEI-PEG-EGF+Mel**



EGFR-targeted poly (I•C): Other EGFR - overexpressing Tumors

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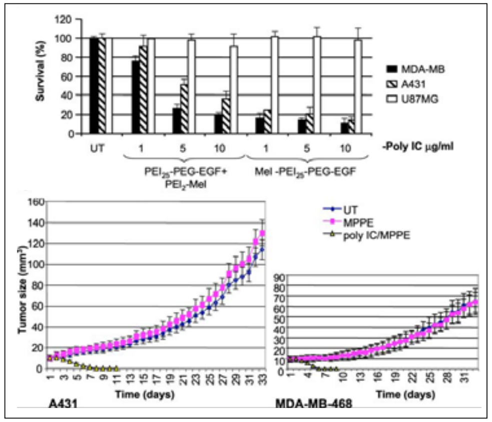
- “Tetra-conjugate“ MPPE more effective than mixture of two conjugates
- Complete elimination of established breast cancer and adenocarcinoma xenografts



W. Rödl, M. Ogris / A. Shir, A. Levitzki *PLOS Medicine* 2006

LMU

Improved Polyplexes:

- Better Defined Polymers
- Better Endosomal Escape
- Extracellular stabilization Intracellular disassembly

GiANT — Gene therapy: an Integrated Approach for Neoplastic Treatment

CeNS — Center for NanoScience Ludwig-Maximilians-Universität

Biodegradable (Ester, Disulfide, Amide-based) Polyamines

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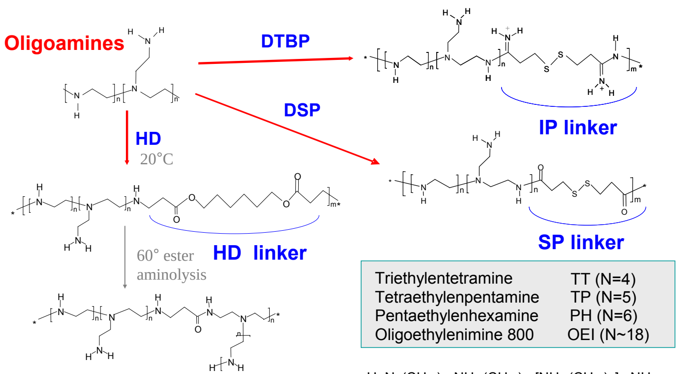


Triethylentetramine	TT (N=4)
Tetraethylenpentamine	TP (N=5)
Pentaethylenhexamine	PH (N=6)
Oligoethylenimine 800	OEI (N~18)

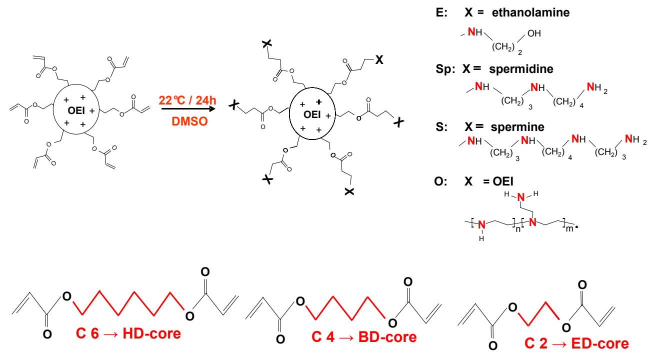
$H_2N\text{-}(CH_2)_3\text{-}NH\text{-}(CH_2)_4\text{-}[NH\text{-}(CH_2)_3]_x\text{-}NH_2$

Spermidine	SD (N=3)
Spermine	SP (N=4)

Julia Klöckner et al *Eur. J. Pharm. Sci* 2006
Peter Tarcha et al *Biomaterials* 2007

Pseudodendritic Polyamines: Better Defined

LMU



Verena Russ et al, *Gene Therapy* 2008

Biocompatibility Studies of HD O *in vivo*

A/J mice, polyplexes in HBG buffer
i.v. application, 50 µg pCMVLuc per mouse

- no necrotic changes in liver tissue
- liver enzyme levels similar to control

	(AST + ALT)/GLDH	AP
buffer	5,3	171
HD O c/p 1	6,3	189
LPEI c/p 0.8	11,7	190
BPEI c/p 0.8	17,3	253

- HDO pseudodendrimer mediates *in vivo* gene transfer to tumor tissue with high biocompatibility

➢ Encouraging starting point for degradable polymers with defined G2 and G3 dendrimer cores

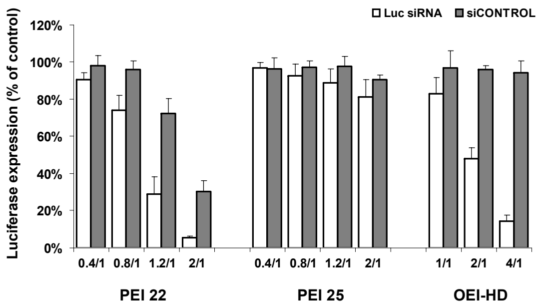
Verena Russ et al, *Gene Therapy* 2008

Tumor targeted siRNA therapy

GiANT
Gene therapy: an Integrated Approach for Neoplastic Treatment

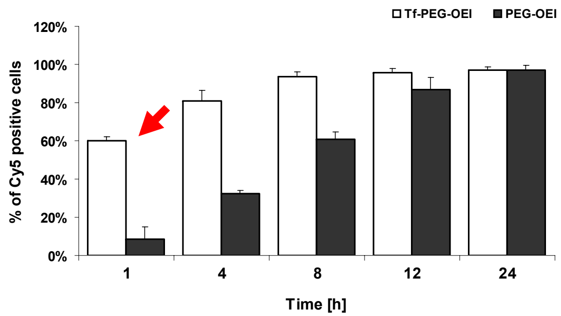
CeNS
Center for NanoScience
Ludwig-Maximilians-Universität

OEI-HD Polyplexes for siRNA Delivery



Nicole Tietze, Jaro Pelisek et al *Oligonucleotides 2008*
Peter Tarcha et al *Biomaterials* 2007
Julia Kloeckner et al *Bioconjug. Chem.* 2006

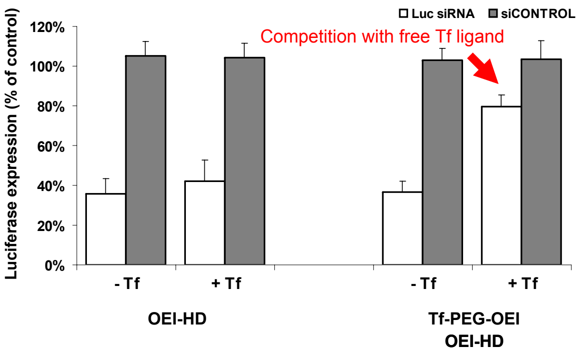
Transferrin-coated OEI-HD Polyplexes for siRNA Delivery into Tumors



Faster internalization by ligand Tf

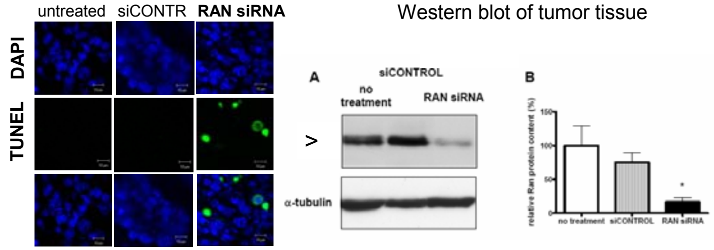
Nicole Tietze, Jaro Pelisek *et al.* *Oligonucleotides 2008*

Transferrin-coated OEI-HD Polyplexes for siRNA Delivery into Neuro2a Tumors



Nicole Tietze, Jaro Pelisek *et al.* *Oligonucleotides 2008*

Transferrin-coated Polyplexes for Systemic siRNA Delivery into Neuro2a Tumors

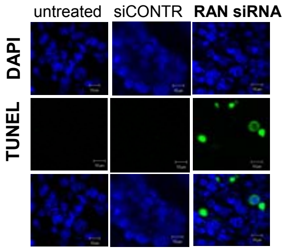


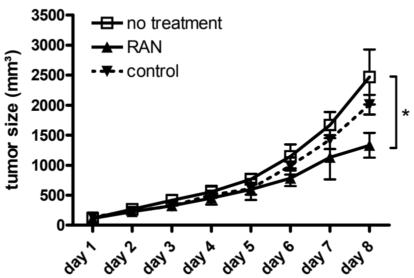
apoptosis in tumor tissue

80% knockdown in tumor tissue

Nicole Tietze, Jaro Pelisek *et al.* *Oligonucleotides 2008*

Transferrin-coated Polyplexes for Systemic siRNA Delivery into Neuro2a Tumors





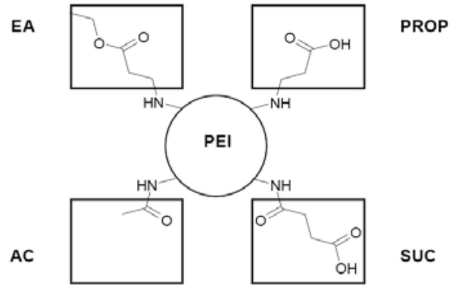
apoptosis in tumor tissue

Nicole Tietze, Jaro Pelisek *et al.* *Oligonucleotides 2008*

Polyethylenimine for siRNA delivery ?

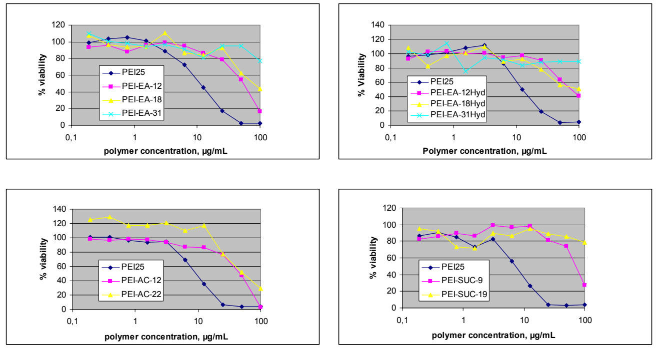
- Moderate efficiency of PEI (Grayson/Putnam 2006, others)
- Depending on type (linear/branched) and size of PEI, e.g. LMW purified branched PEI (Aigner 2006)
- *In vitro* vs. *in vivo* efficiency
- Stability issue (→ sticky siRNA, Bolcato Bellemin/Behr 2007)
- Intracellular release issue (LMW PEI for mRNA delivery, Bettinger 2001)

siRNA Delivery: Simple bPEI 25kDa Derivatives



Arkadi Zintchenko et al *Biocon. Chem. 2008*

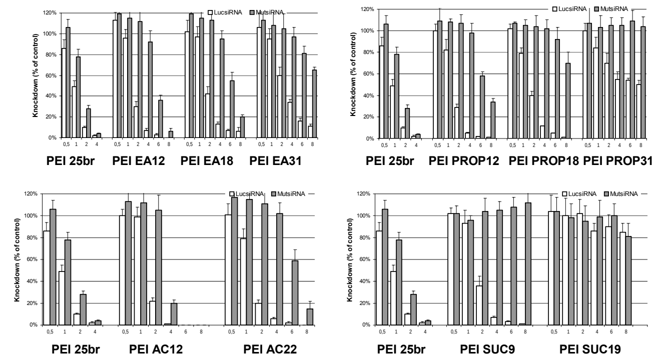
Reduced Cytotoxicity of Modified Polymers



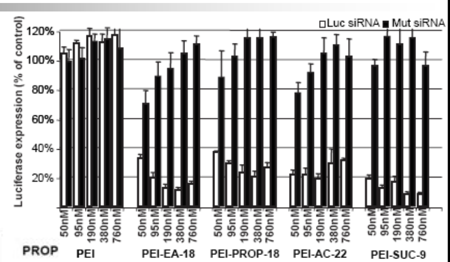
Arkadi Zintchenko et al *Biocon. Chem. 2008*

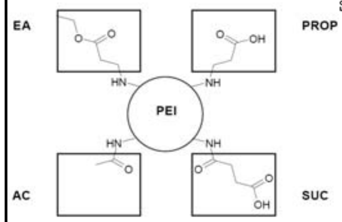
Neuro2A cells

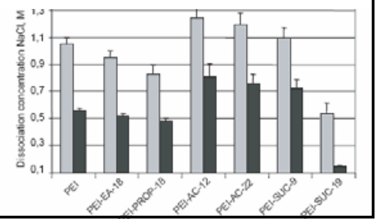
siRNA knockdown Neuro2ALuc



siRNA Delivery: Simple bPEI Derivatives







Arkadi Zintchenko et al *Biocon. Chem. 2008*

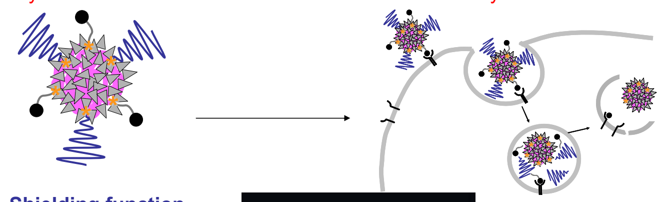
Improved Polyplexes:

- Better Defined Polymers
- Better Endosomal Escape
- Extracellular stabilization
  Intracellular disassembly

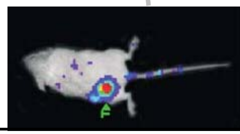
Programmed Nanosystems – 'Synthetic Viruses'

- Pre-programming to alter their properties during drug delivery

Chemical / Physical — Molecular Sensor — Responding to — Biological / Physical — Trigger



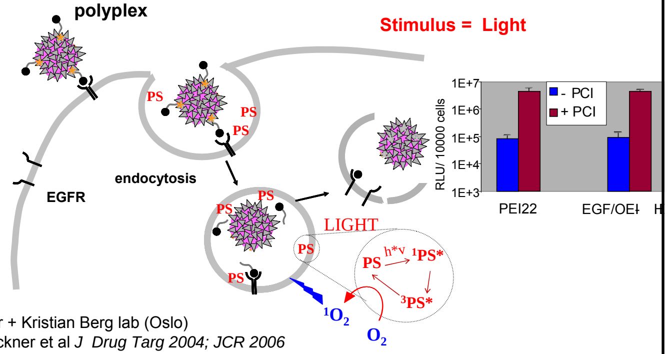
Shielding function (blood circulation)
Targeting function
Triggered release (at target site/cell)



Photochemical Internalization (PCI): Improved Endosomal Release

- Sensor = Photosensitizer   Trigger = Light

Sensor = amphiphilic photosensitizer (PS)
Stimulus = Light



Wagner + Kristian Berg lab (Oslo)
J. Kloeckner et al *J Drug Targ 2004; JCR 2006*

Photochemical Internalization (PCI): Improved Endosomal Release

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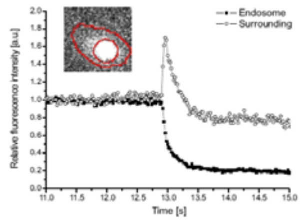
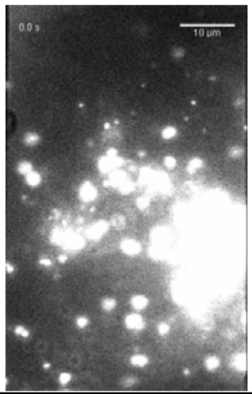
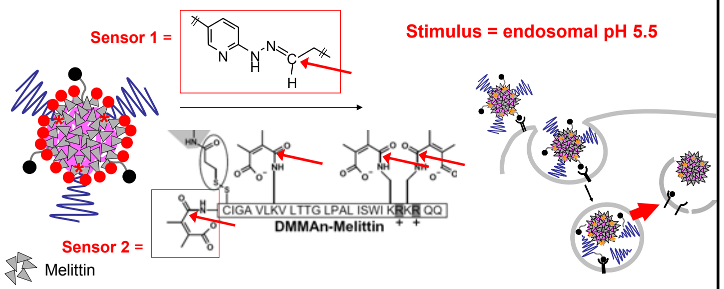




Nadia Ruthardt, Karla de Bruin et al *JCR 2008*
CeNS collaboration Christoph Bräuchle lab

Dynamic Polymers : PEG Deshielding / Endosomal Escape

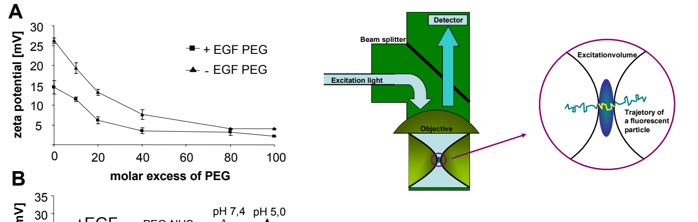
• Sensors = pH-labile bonds Trigger = endosomal pH



pLL, PEI
polyplexes

Walker *Mol. Ther. 2005*
Fella EJPS 2008
Meyer *JGM 2007*

pH-reversible bifunctional PEG : HZN Linkage

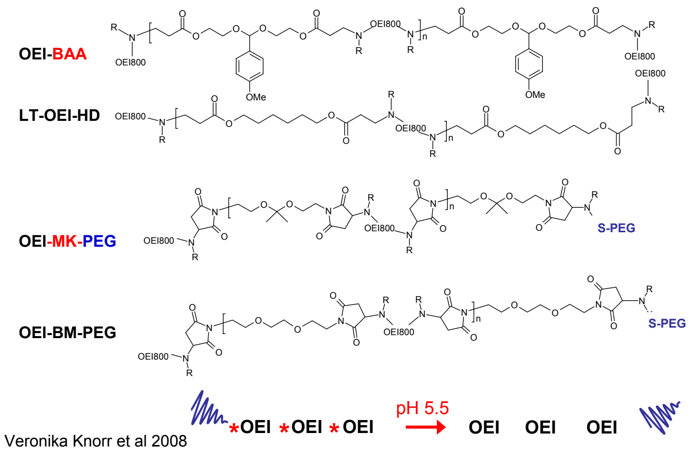


Diffusion times by fluorescence correlation spectroscopy (FCS)

	pH	Diffusion time (µs)
Cysteamine-modified Alexa-NHS	7.4	31 +/- 5
Alexa-SS-PEG-NHS	7.4	193 +/- 18
Alexa-SS-PEG-HZN-NHS	7.4	196 +/- 21
DNA/BPEI, Alexa-SS-PEG-NHS	7.4	1826 +/- 560
	5.0	1988 +/- 1209
DNA/BPEI, Alexa-SS-PEG-HZN-NHS	7.4	1713 +/- 314
	5.0	192 +/- 19

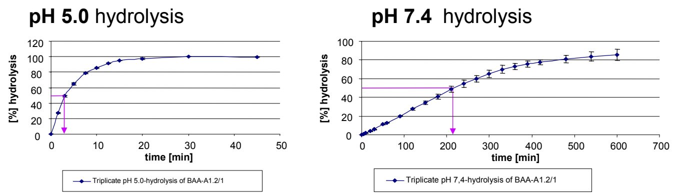
C. Fella et al *EJPS 2008*

Acid-labile Acetal vs Acid-stable Polymers



Veronika Knorr et al 2008

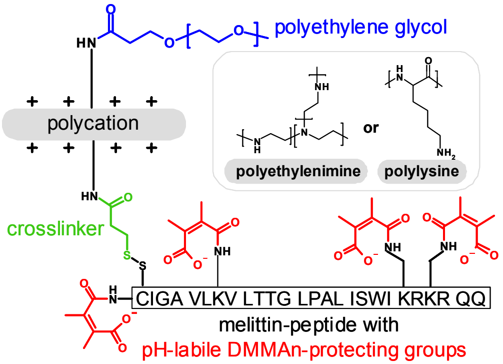
Degradabile Acetal Polymers



OEI-**BAA**:	OEI-**MK**:
pH **7.4** ~ 3 ½ h (at 37°C)	pH **7.4** ~ 5 h (at 37°C)
pH **5.0** ~3 min	pH **5.0** degraded after 15 min

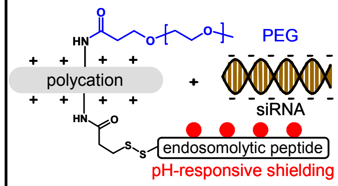
Veronika Knorr et al *Bioconjug. Chem. 2008*

siRNA Delivery: pH-responsive endosomolytic peptides & PEG

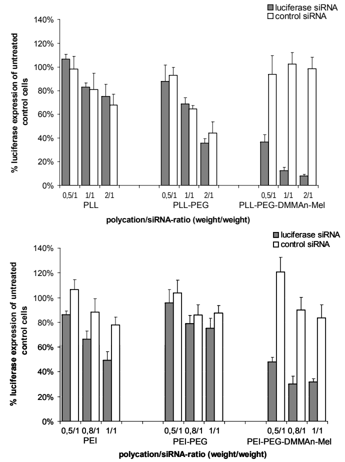


Martin Meyer et al *JACS 2008*

siRNA Delivery: pH-responsive endosomolytic peptides & PEG



Luciferase knock-down in Neuro2A-Luc cells



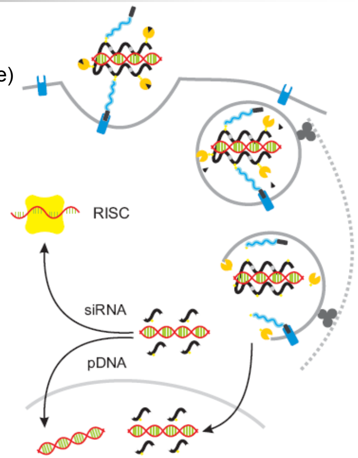
Martin Meyer et al *JACS 2008*

Conclusions and Prospects

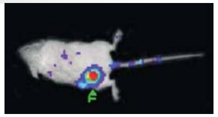
Schaffert & Wagner, *Gene Therapy 2008*
Gene therapy progress and prospects: synthetic polymer-based systems

Intracellular Pathways and *in vitro* Barriers

- Active targeting for enhanced cellular uptake
  (Shir/ deBruin/Rozema/Barlett/Tietze)
- Reversible PEG shielding
  (Walker/Knorr/Fella, Stayton, Rozema, Kataoka, Szoka, Torchilin, others)
- Bioresponsive backbone for siRNA release
  (ester, disulfide, acetal, others)
- Triggered endosome escape
  (Plank,Ogris, Rozema, others)



Extracellular Pathways and *in vivo* Barriers



- Avoid unspecific interactions
  - shielding (PEG, various molecules)
- Avoid extracellular polyplex dissociation
  (e.g. Burke/Pun 2008 ; Buyens JCR 2008)
  - sticky ssiRNA (Bolcato-Bellemin/Behr 2007)
  - bioreversible lateral stabilization (Oupicky 2001, Neu 2007)
  - bioreversible covalent conjugates (Rozema/Wolff 2007)
- Avoid siRNA degradation
  - chemically stabilized siRNA
  - protective polymers
- Reduce toxicity
  - biodegradable, balanced systems

Polymers for siRNA – Chemistry & Development

- Dynamic, bioresponsive 'programmed' polymers which adapt to delivery barriers

  - extracellular stabilization
  - intracellular disassembly

- Improved chemistry will provide

  - monodisperse polymers with uniform size and topology (solid-phase, dendrons, dendrimers)

  - multifunctional sequence defined polymers
  (e.g. Hartmann/Börner 2008)