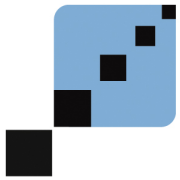
Sequence-defined oligomers for intracellular delivery of nucleic acids, drugs, proteins

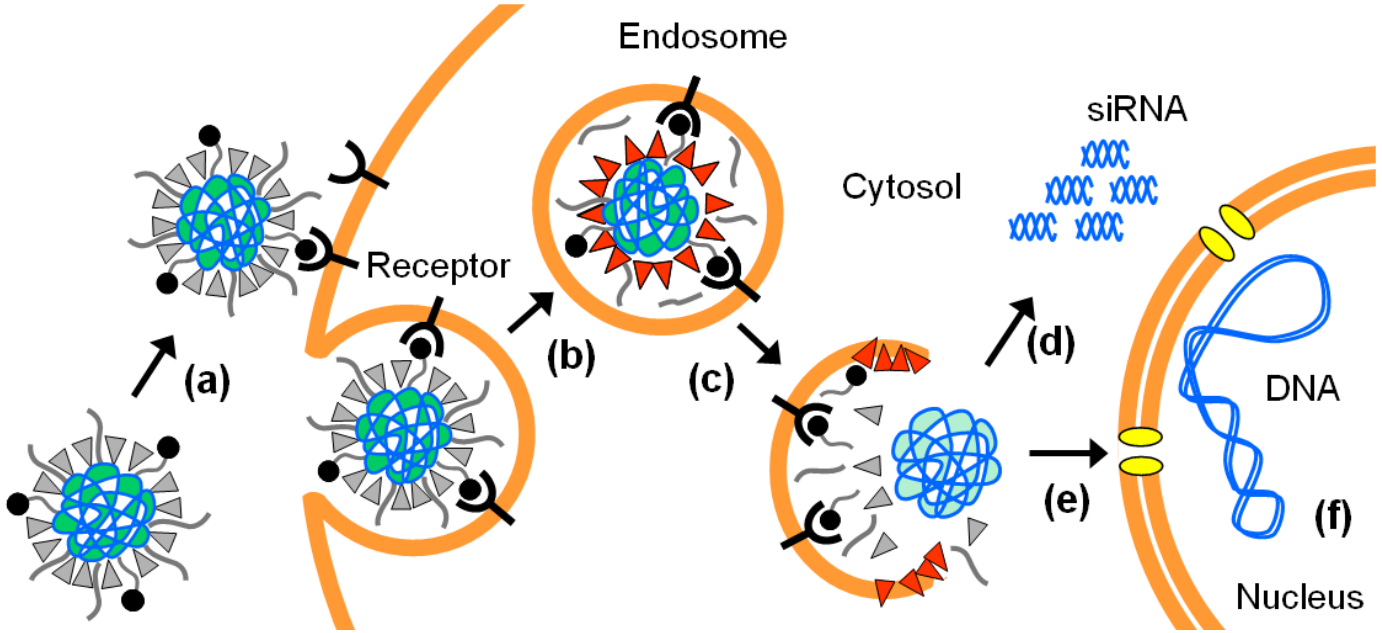


CeNS

Center for NanoScience | LMU

al and artificial oligo-amino acids

22 - 09 - 2015



Bioresponsive carriers

- **multifunctional** and **precise**

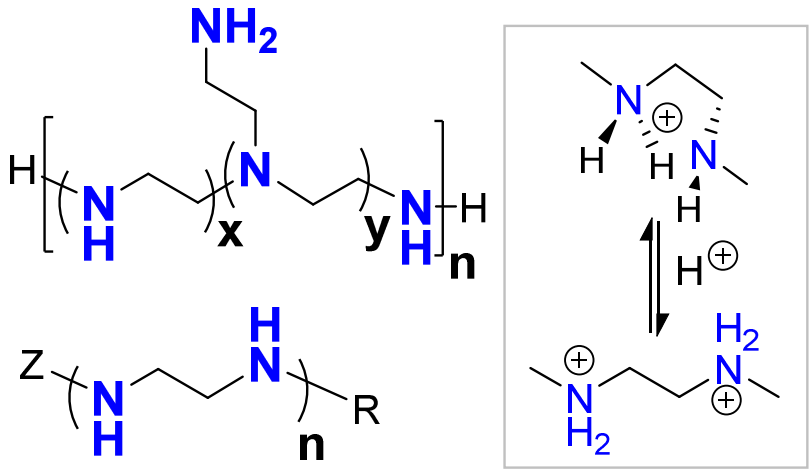
Strategy: learn from viruses and natural evolution

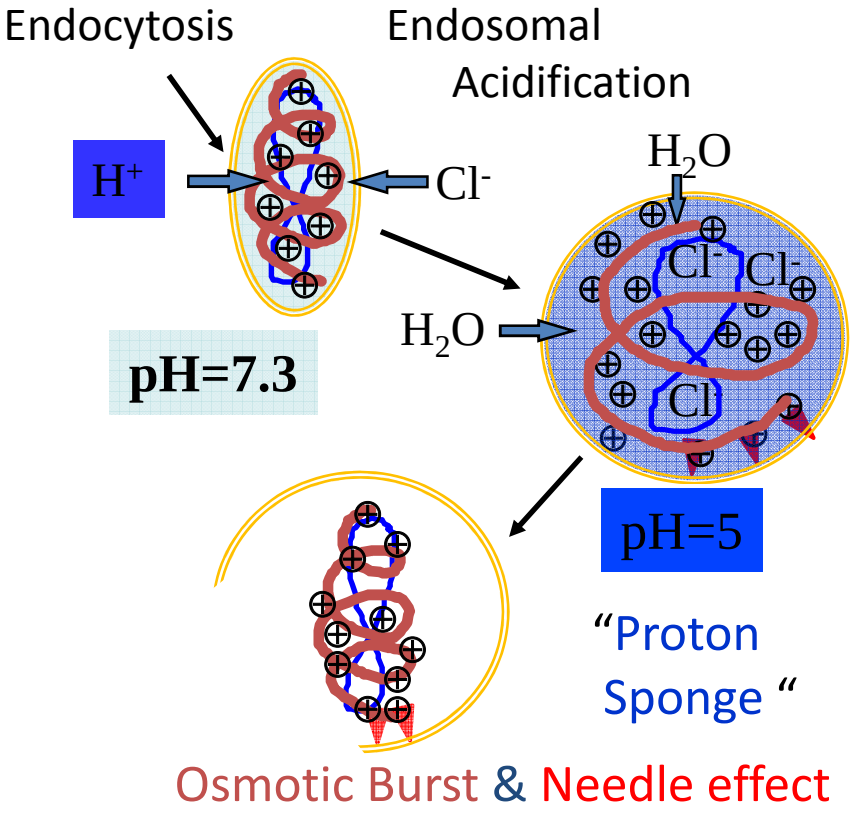
> **Chemical evolution:**

- identify **chemical motifs** for delivery
- assemble chemical **motifs** into **defined sequences**
- shuffle into various **topologies**
- optimize **nanoparticle assembly**

Identify chemical motifs for delivery:

pH-responsive diamino ethane motif





PEI branched or linear
(Boussif et al, JP Behr 1995)

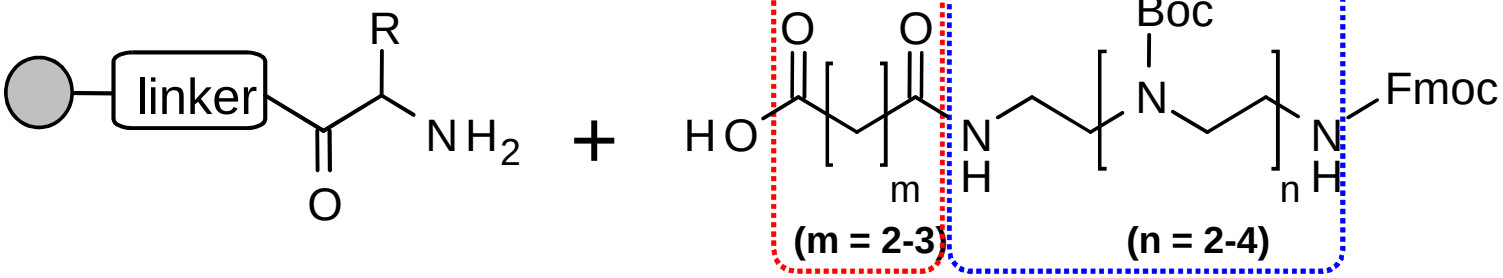
PEI is nondegradable & toxic

Other delivery motifs:

His, KHH; C; CXC; $OleA_2$, $LinA_2$; Y_3; INF7; TAT; receptor ligands

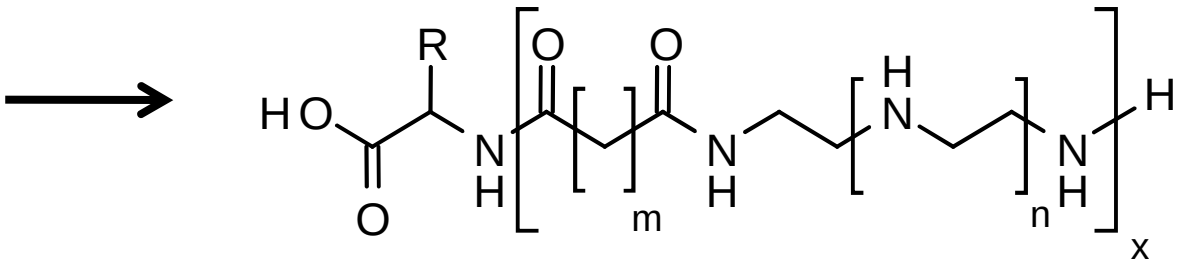
Ulrich Lächelt *Chemical Reviews 2015*
Nucleic Acid Therapeutics Using Polyplexes – A Journey of 50 Years (and Beyond).

Solid phase supported polymer synthesis



Compare:
Laura Hartmann, Hans Börner
Sequence-defined precision polymers

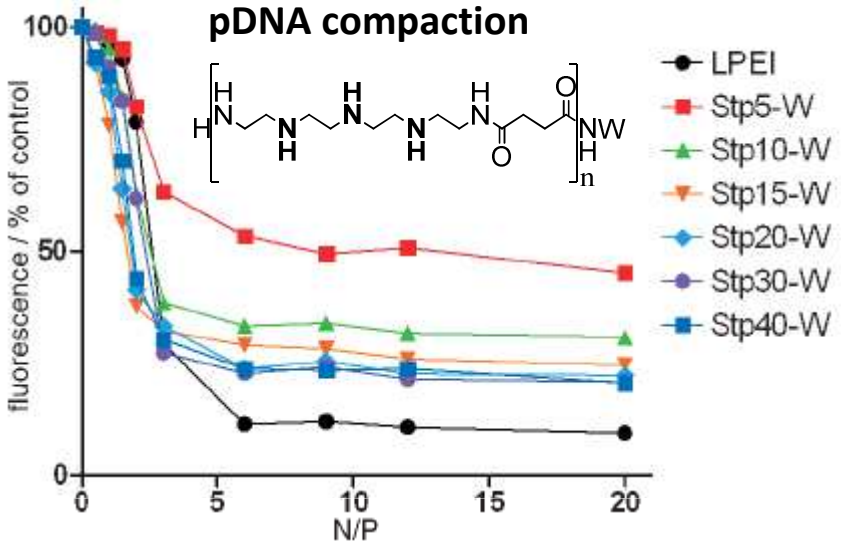
'Artificial oligoamino acids'
(diaminoethane motifs)

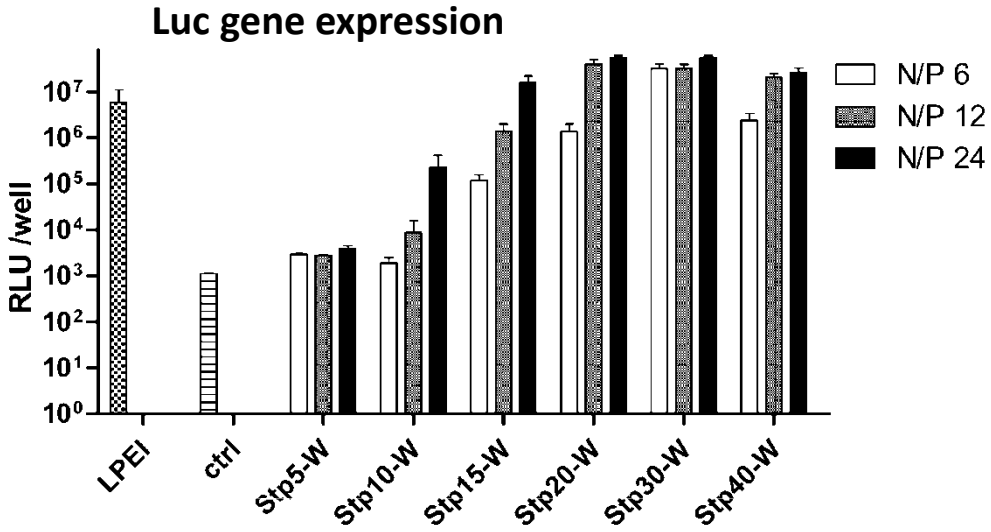


> 900 sequences;
different topologies
excellent biocompatibility

David Schaffert *Org Lett* 2011, *Angewandte Chemie 2011, Bioconjug Chem 2012*

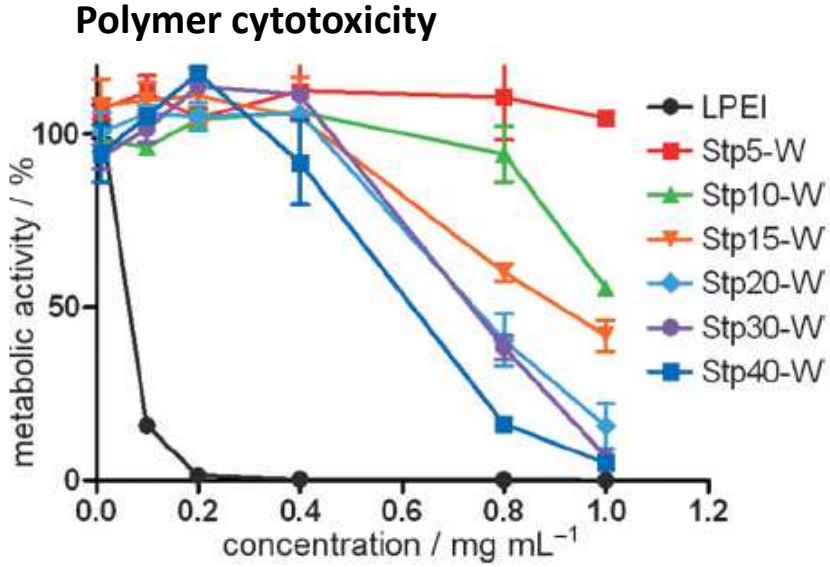
Correlation of polymer size with gene transfer and cytotoxicity





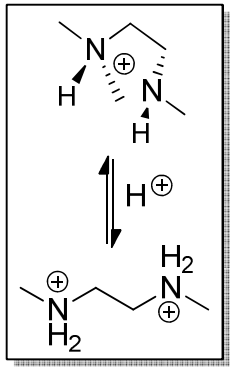
LPEI 22kDa: approx 500 (±200) nitrogens

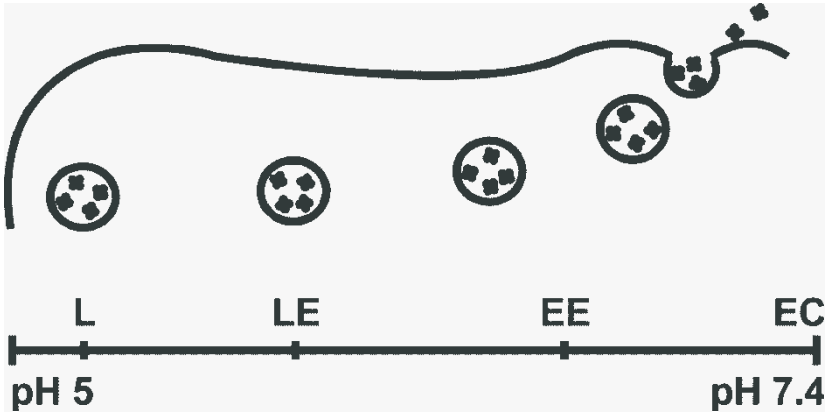
Stp_{30}: 91 amine nitrogens (150 nitrogens)

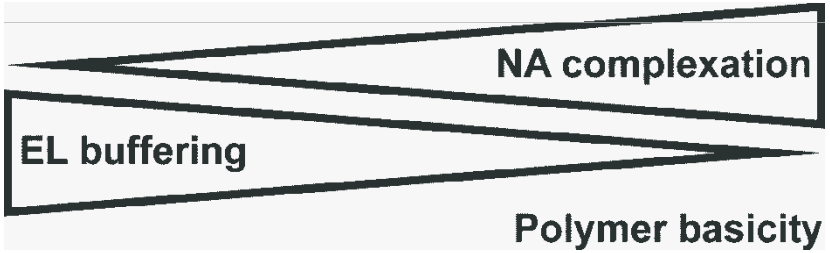


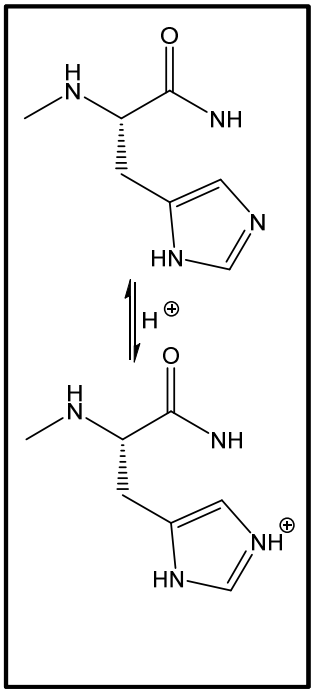
Claudia Scholz, Petra Kos et al *ChemMedChem* 2014

Diaminoethane and histidine motif:
Fine-tuning of proton sponges







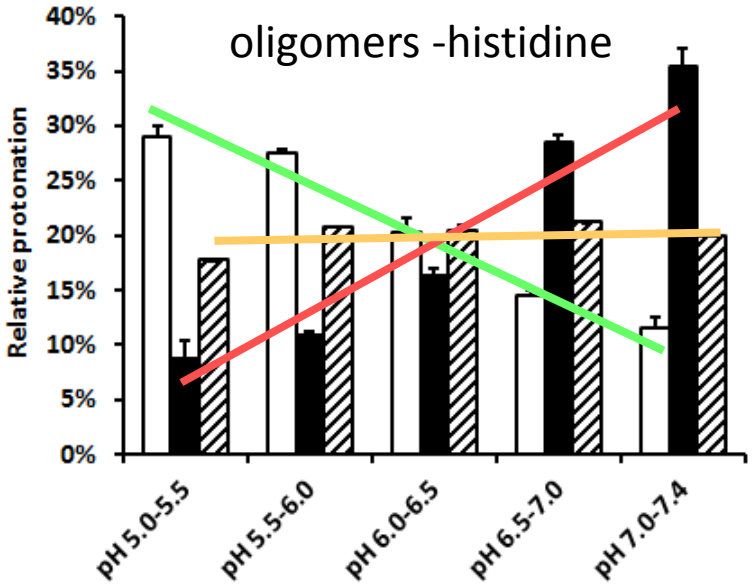


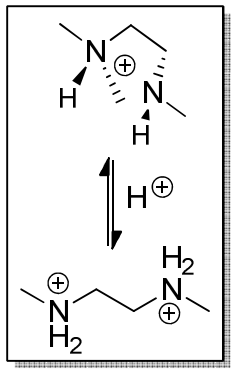
EC: extracellular
EE: early endosomes
LE: late endosomes
L: lysosomes
NA: nucleic acid

Ulrich Lächelt et al *Nanomedicine NBM* 2014

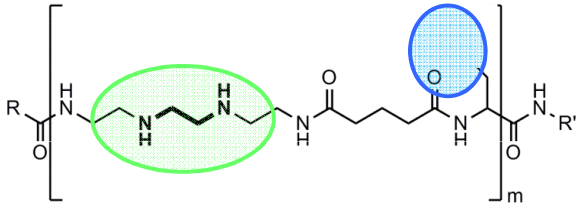
Fine-tuning of proton sponges:

Protonation in endosomal pH range

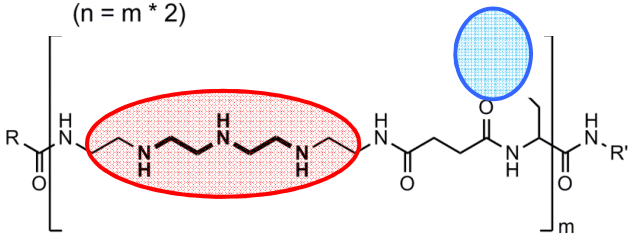




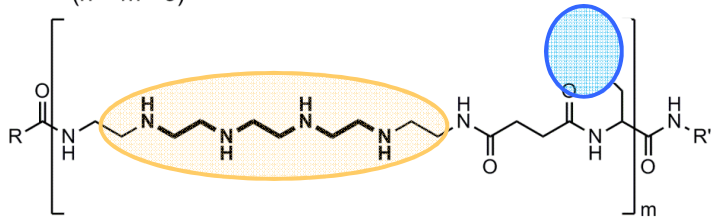
	Buffer capacity (pH 5-7.4)
610 / A-(GTT-A)3	31.7 ± 1.7%
611 / H-(GTT-H)3	33.6 ± 0.5%
612 / A-(STP-A)3	14.7 ± 0.6%
613 / H-(STP-H)3	22.4 ± 0.9%
614 / A-(SPH-A)3	23.6 ± 2.1%
615 / H-(SPH-H)3	27.9 ± 1.3%



$(GTT\text{-}H)_m$
(n = m * 2)

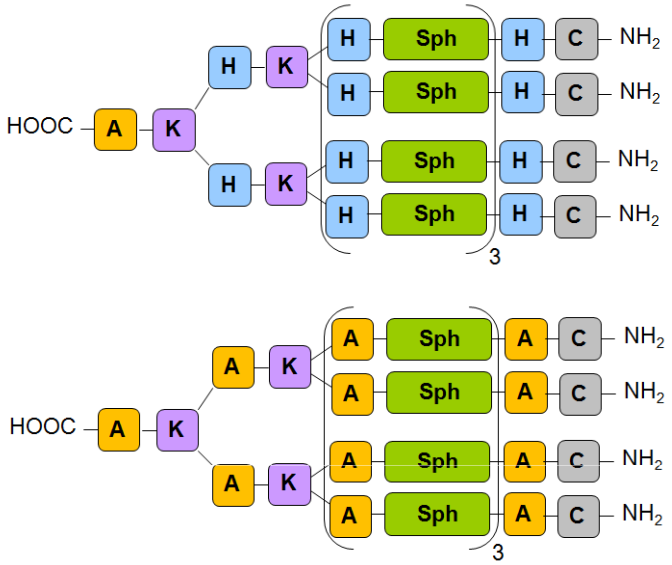


$(STP\text{-}H)_m$
(n = m * 3)

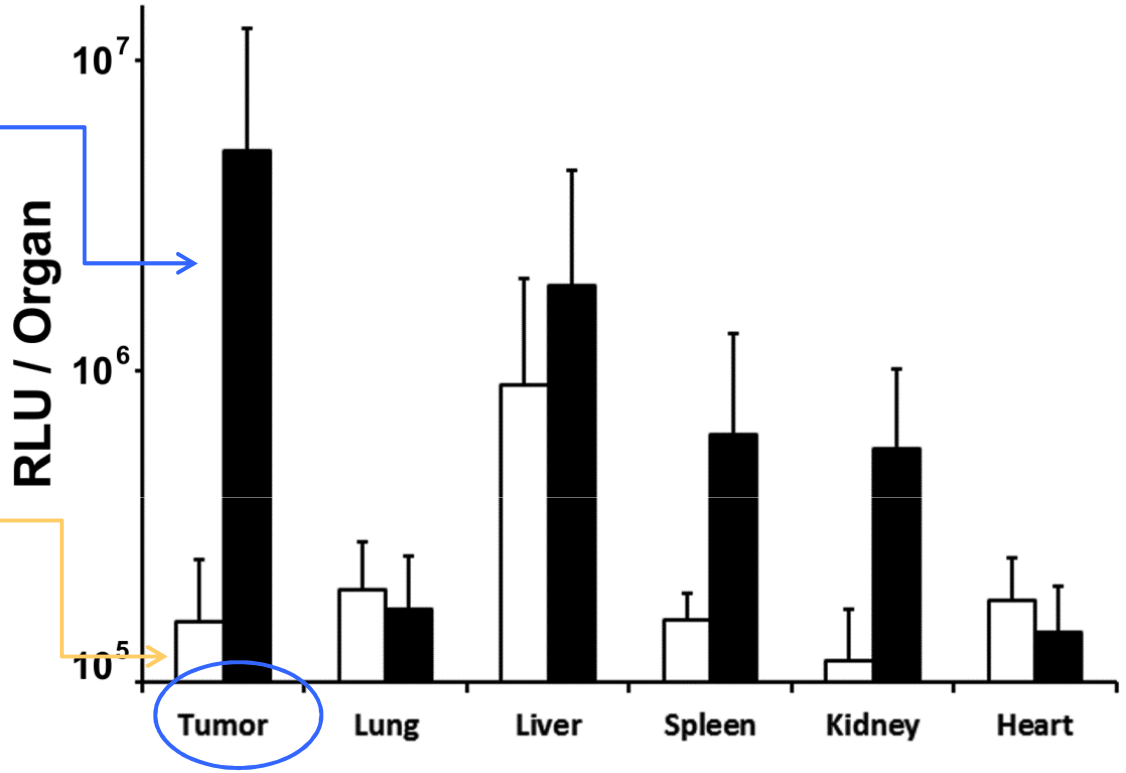


$(SPH\text{-}H)_m$
(n = m*4)

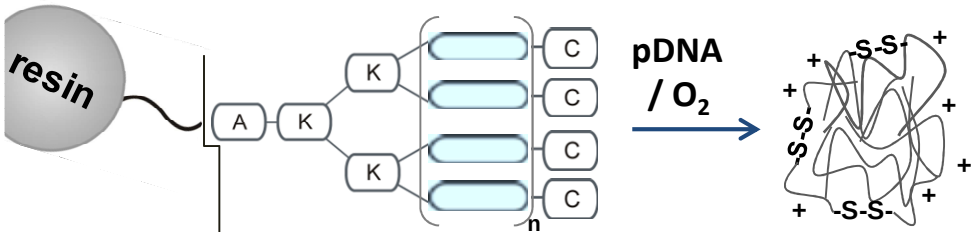
Four-arms containing histidine: Systemic delivery in sc N2A tumor model



pDNA polyplexes N/P 12



His-Sph 4-arm: 30-fold enhanced gene transfer into tumor



Ulrich Lächelt, Petra Kos, Annika Herrmann *Nanomedicine NBM* 2014

Targeted PEG-shielded polyplexes

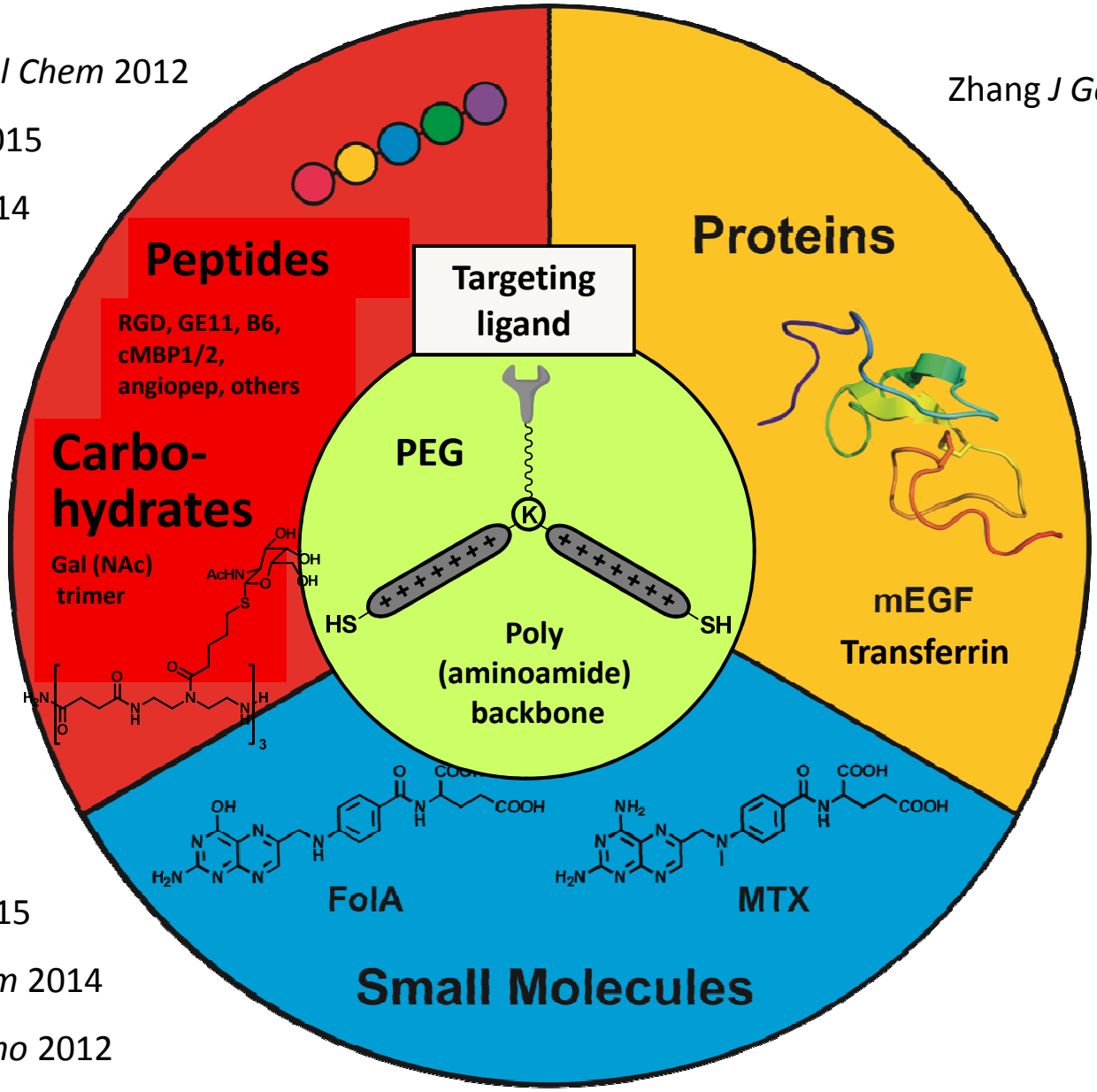
Martin *Org Biomol Chem* 2012

Kos *J Pharm Sci* 2015

Kos *Nanoscale* 2014

An *Small* 2015

Zhang *J Gene Med* 2015

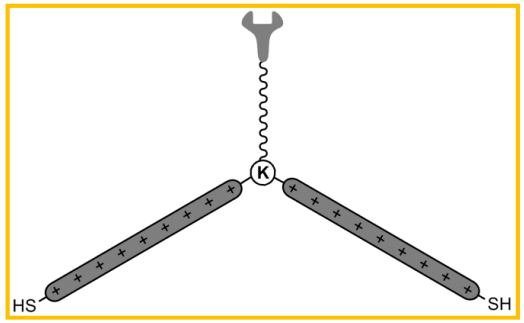


Lee *submitted* 2015

Lächelt *Mol Pharm* 2014

Dohmen *ACS Nano* 2012

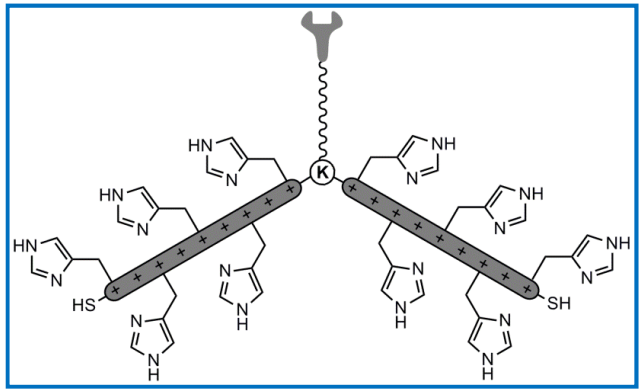
Targeted PEG-shielded polyplexes: Histidines enhance endosomal escape

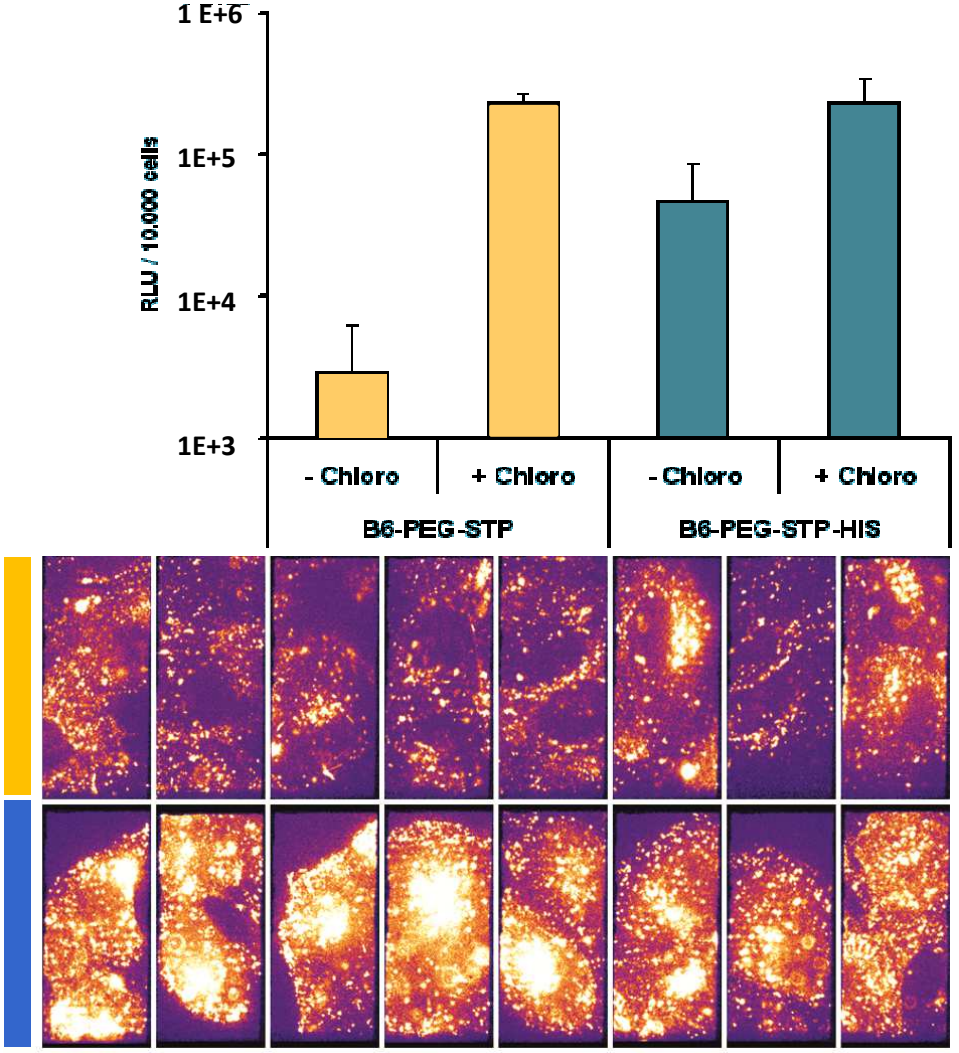


Ligand-PEG-STP-**Ala**/**His**

PEG: 24 EO units

STP arms: 24 EI units





Ulrich Lächelt *Nanomedicine NBM* 2014

Bräuchle lab: polyplexes plus calcein (3.5 h, SD-CFLM)

Ellen Broda *JCR 2015*: cell binding under flow: B6 not via TfR ! cMBP2 binds via c-Met

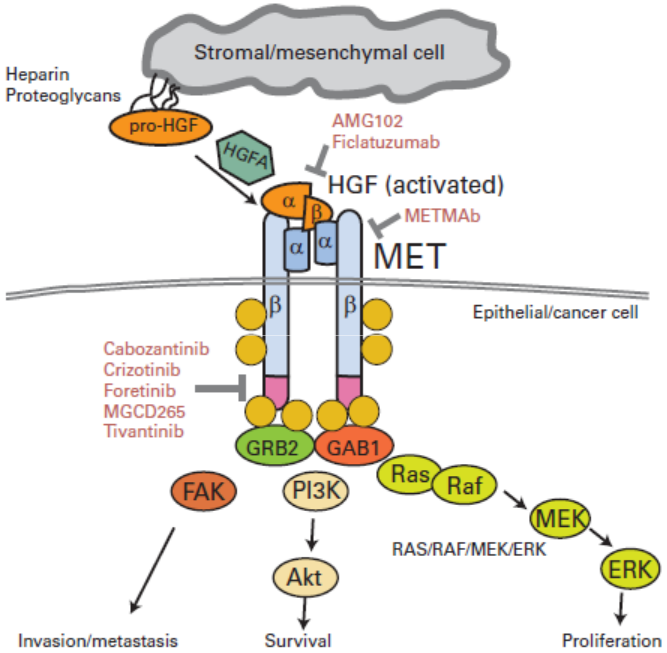
New targeting ligand:
c-Met Binding Peptide (cMBP2)

c-Met / Hepatocyte growth factor receptor

Tyrosine kinase

Proliferation & progression of malignancies

High expression in late stage and metastases of prostate cancer



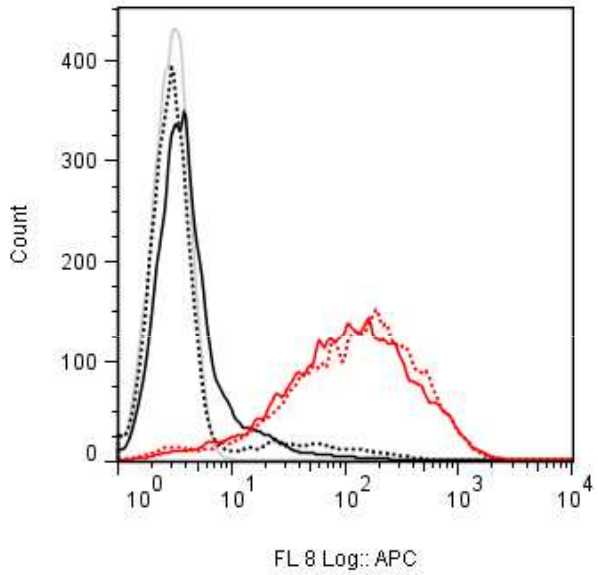
L. Appleman, *JCO* 2011;29: 4837

c-Met binding peptides (cMBP1, cMBP2)

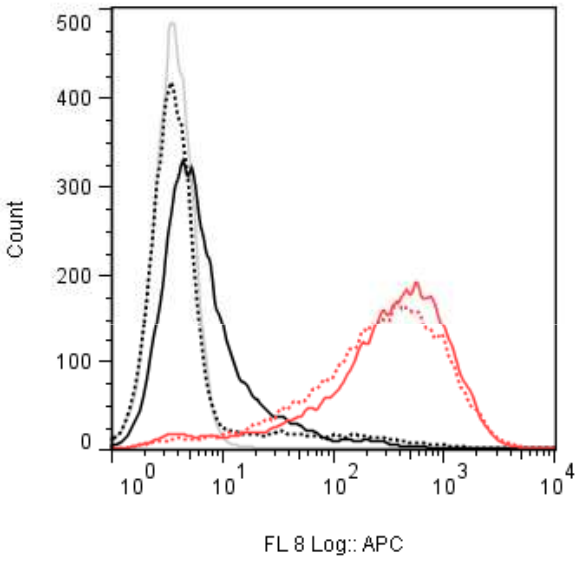
Petra Kos, Uli Lächelt, Annika Herrmann, Dongsheng He *Nanoscale* 2015

Cellular internalization (c-Met targeting)

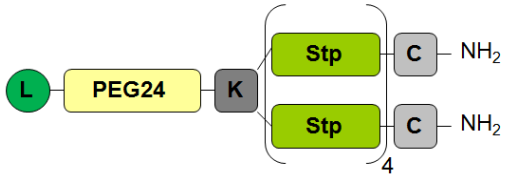
DU-145
prostate ca



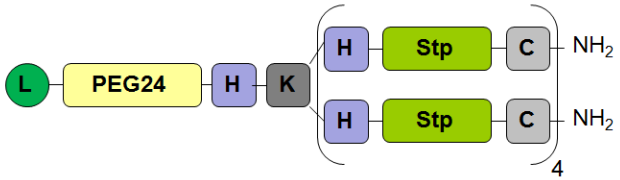
HUH-7
hepatocellular ca



Ala-PEG-STP **cMBP2-PEG-STP**



Ala-PEG-STP-His (dotted) **cMBP2-PEG-STP-His (dotted)**

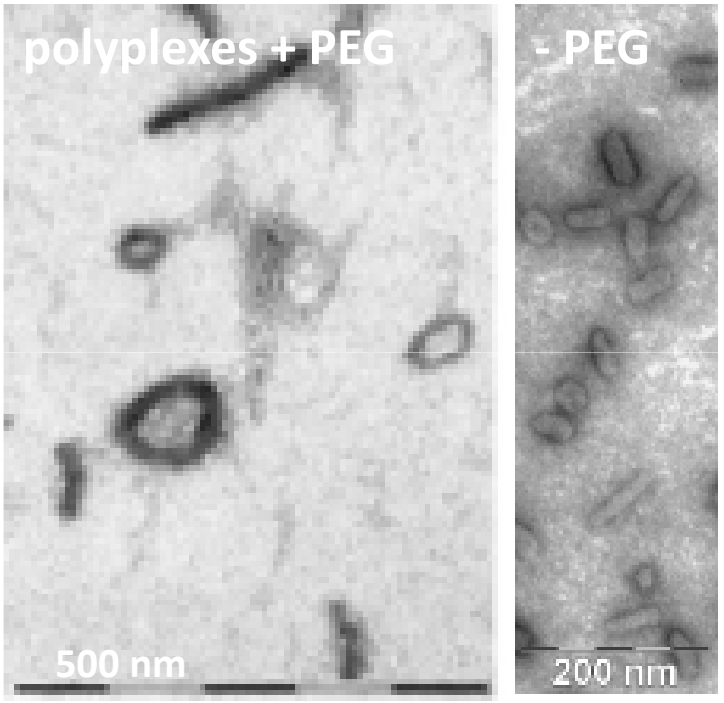


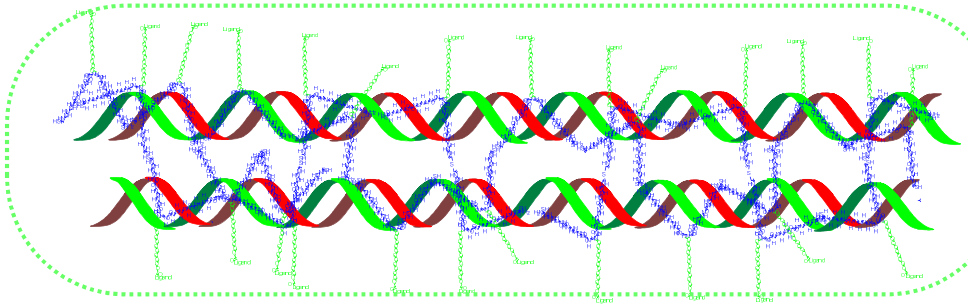
Petra Kos, Uli Lächelt, Annika Herrmann et al *Nanoscale* 2015

c-Met binding peptide for tumor targeting
Systemic delivery in HUH7 tumor model

In vitro and intratumoral: nice targeting, His effect

Systemic: moderate activity, no targeting effect

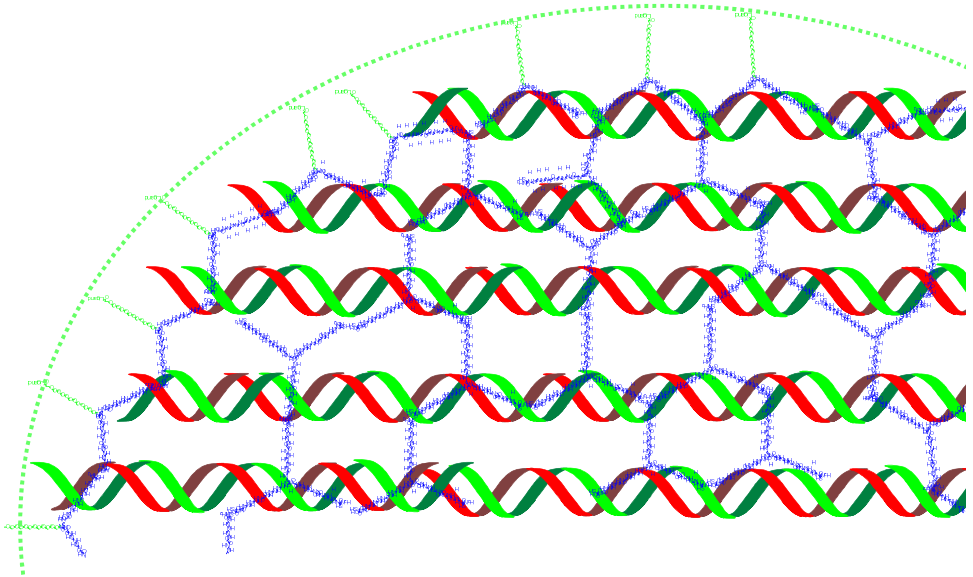




PEG segments

shield surface, but interfere with

DNA - DNA **compaction**

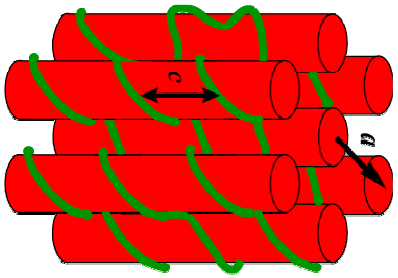


hexagonal

columnar phase

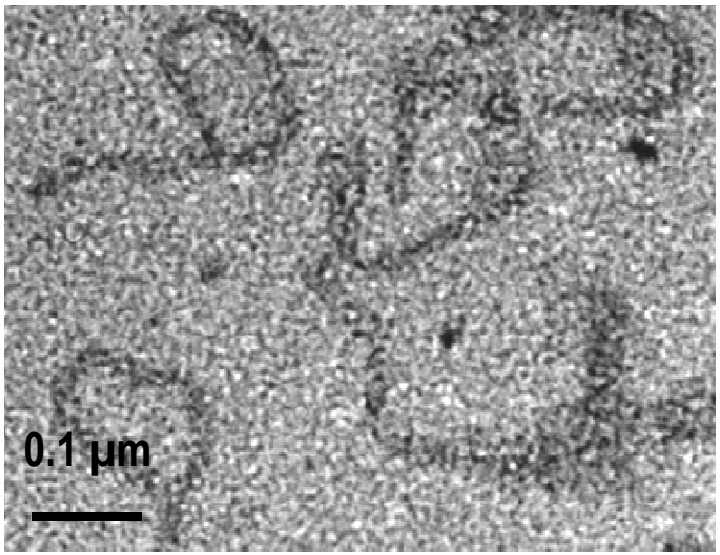
Derouchey, Netz,Rädler

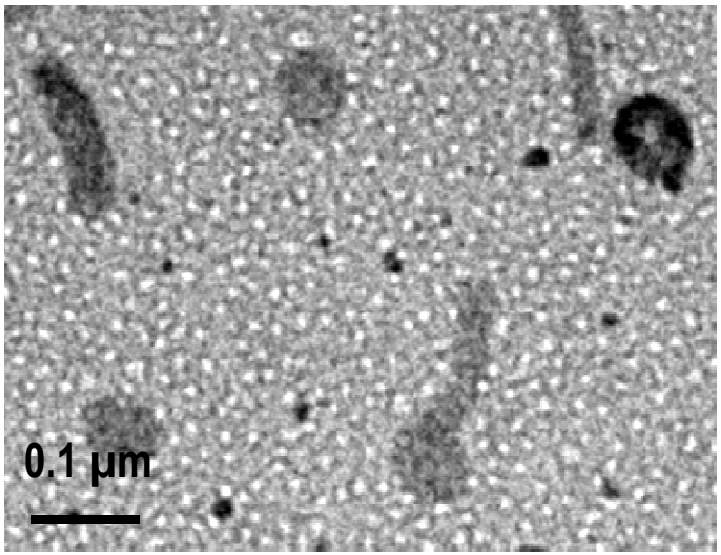
EPJE 2005



c-Met binding peptide for tumor targeting
Systemic delivery in HUH7 tumor model

N/P 12; mixture 7:3 ratio





Petra Kos, Uli Lächelt, Annika Herrmann et al *Nanoscale* 2015

c-Met binding peptide for tumor targeting
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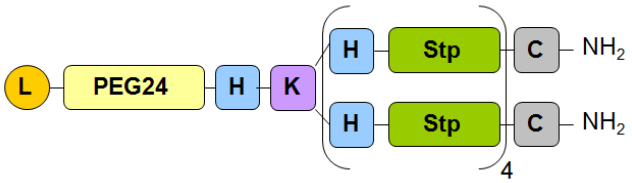

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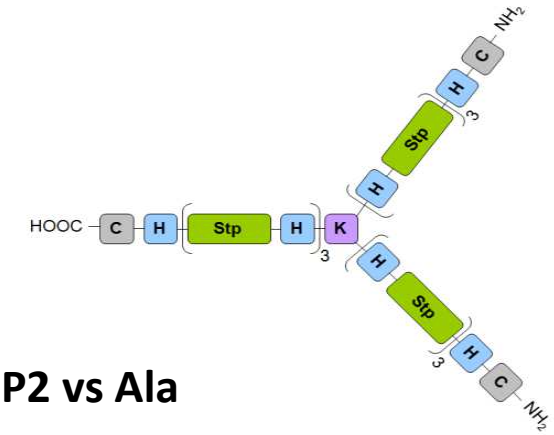
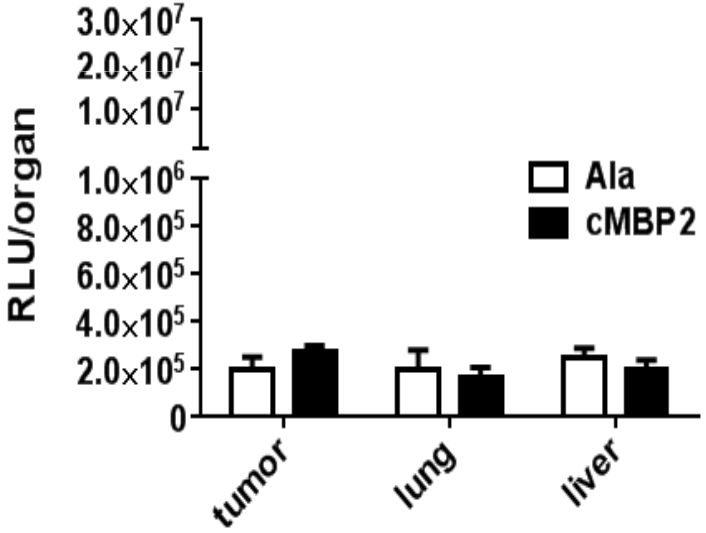
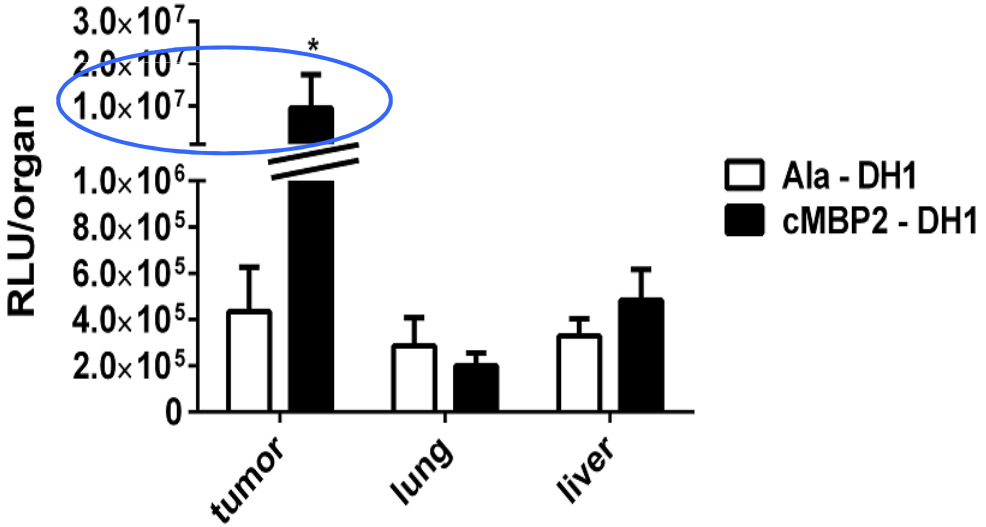


cMBP2 vs Ala



Mix: cMBP2 vs Ala

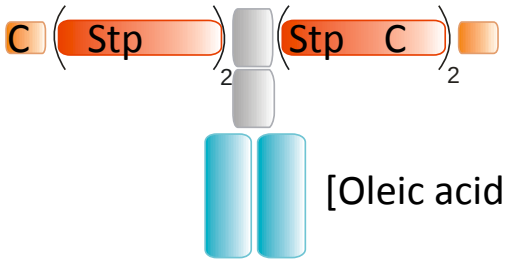


cMBP plus His: 50-fold enhanced gene transfer

Petra Kos, Uli Lächelt, Annika Herrmann et al *Nanoscale* 2015

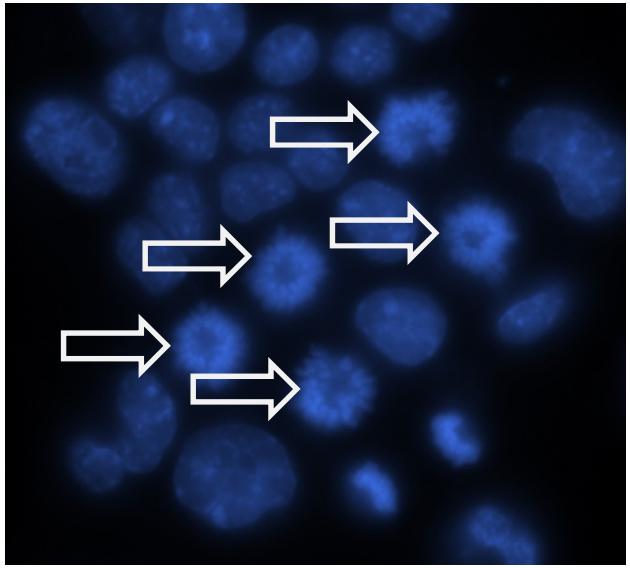
OleA2 – NP stabilizing and lytic motif :

EG5 (Eglin-5, KSP) knockdown by siRNA

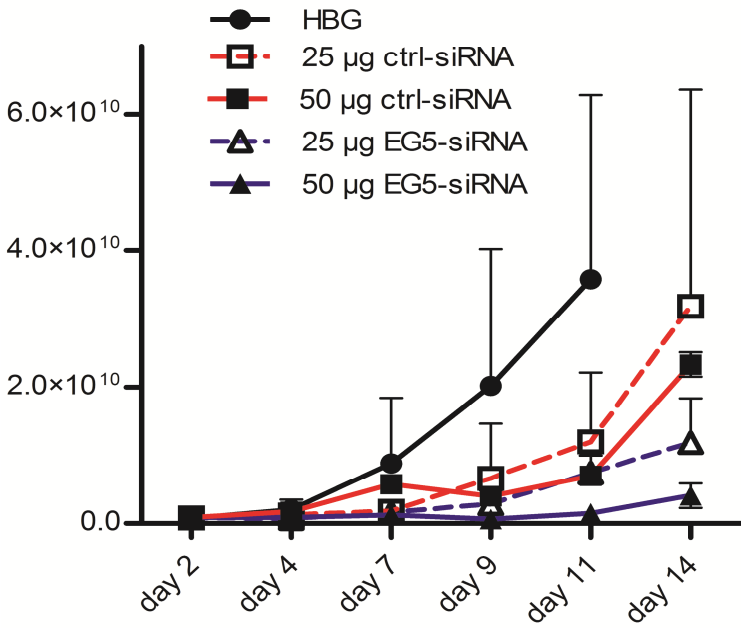


EG5 siRNA / T-shape lipo-oligomer **49**
(polyplexes stable for weeks at RT)

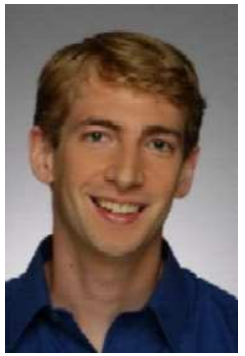
Mitotic aster formation



Tumor growth reduction



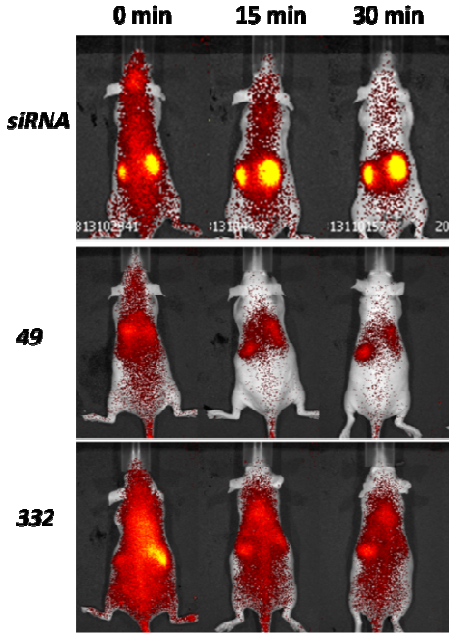
N2A tumors, intratumoral administrations



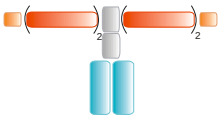
T. Fröhlich et al *JCR 2012* D. Edinger et al *DDTR 2013*

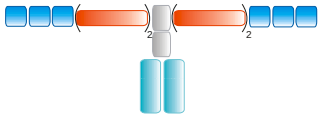
Polyplex stabilizing motifs:

Tyrosine trimer (Y3) and CXC



Free Cy7-siRNA

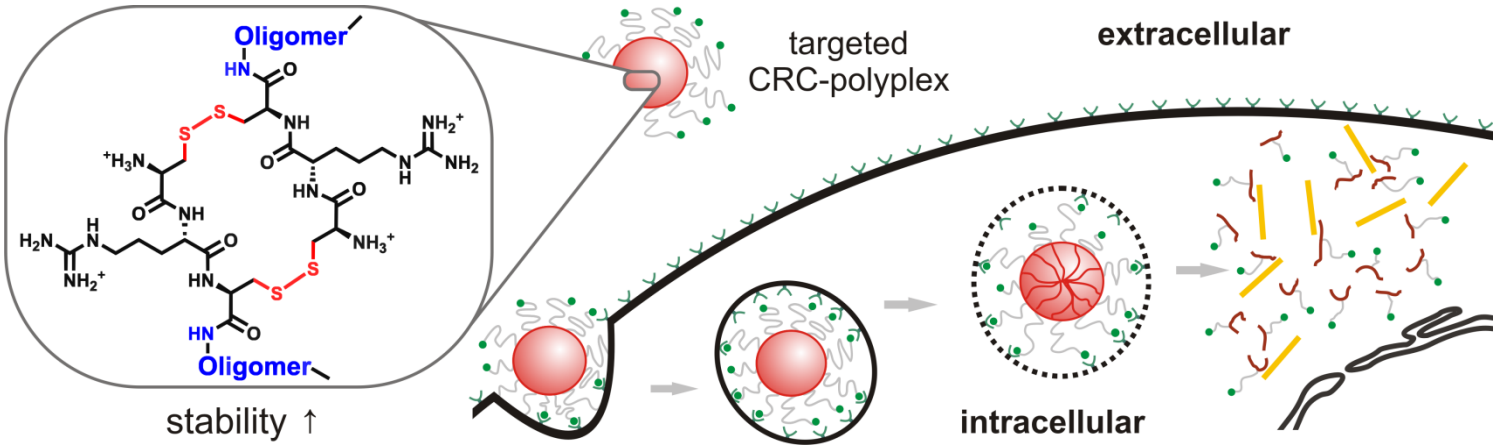




Y_3-Stp_2-[$(OleA)_2$-K]K-Stp_2-Y_3

Christina Troiber
Biomaterials 2013

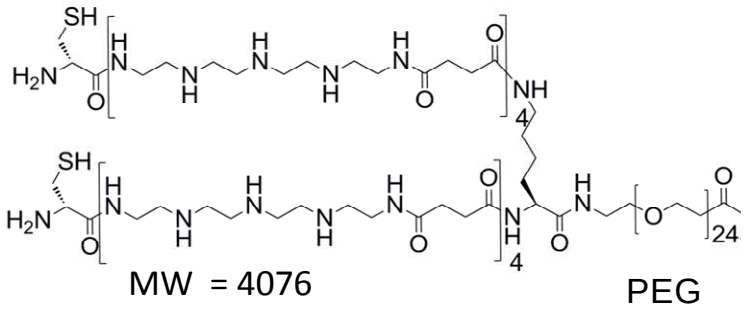
intravenous administration correlates well with *ex vivo* Fluorescence Correlation Spectroscopy in 90% serum



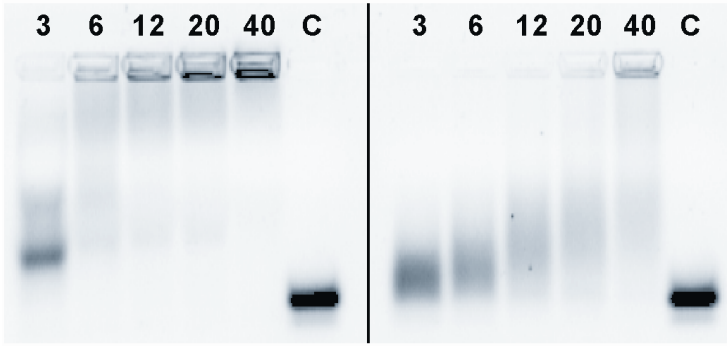
Philipp Klein et al *JCR* 2015 (JC Leroux, M. Gauthier ETHZ)

Folate for targeting:

Multifunctional siRNA polyplexes

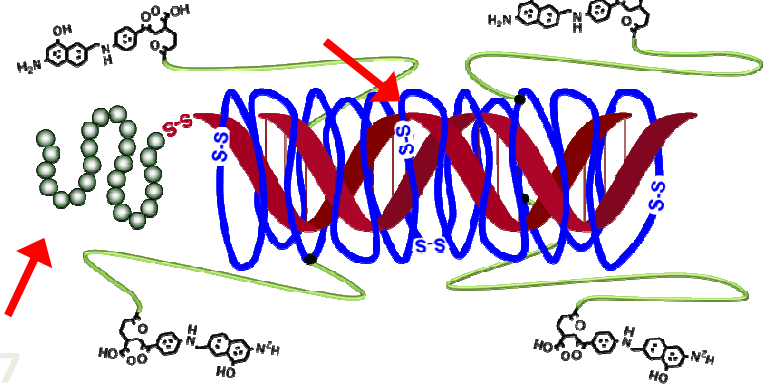


FolA



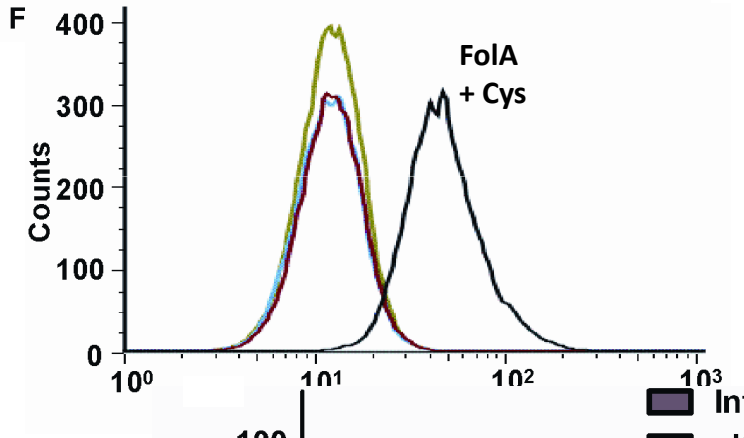
Cys for polyplex stability

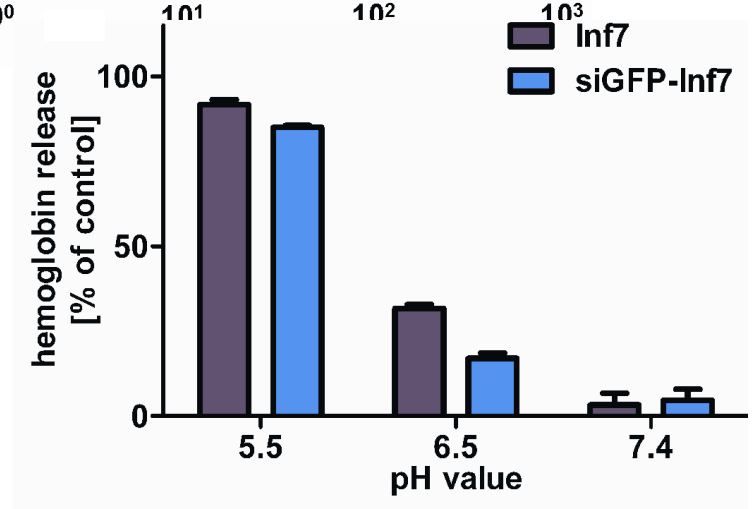
FolA for cellular uptake



Inf7

(influenza peptide) for endosomal escape





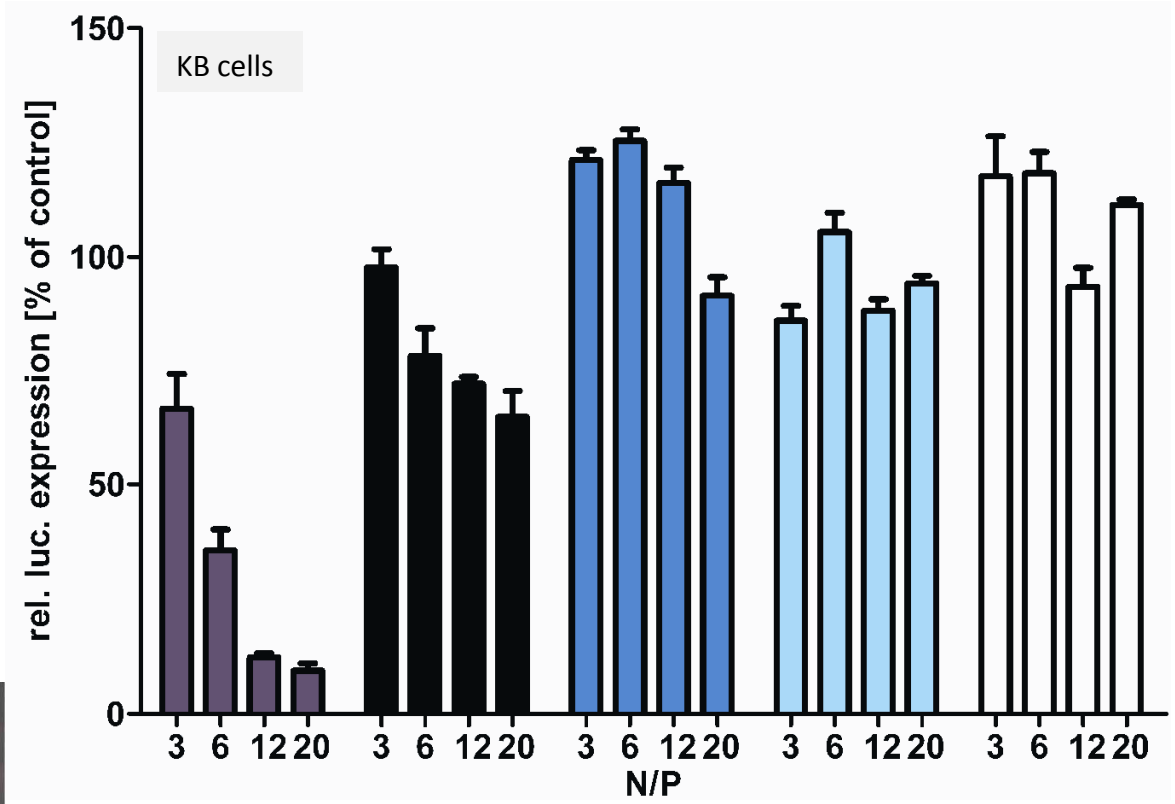
Diameter (PEG-24) **5.8 (±0.2) nm**

siRNA uncomplexed 4.2 (±0.2) nm

Zeta potential **0.0 (±2.4) mV**

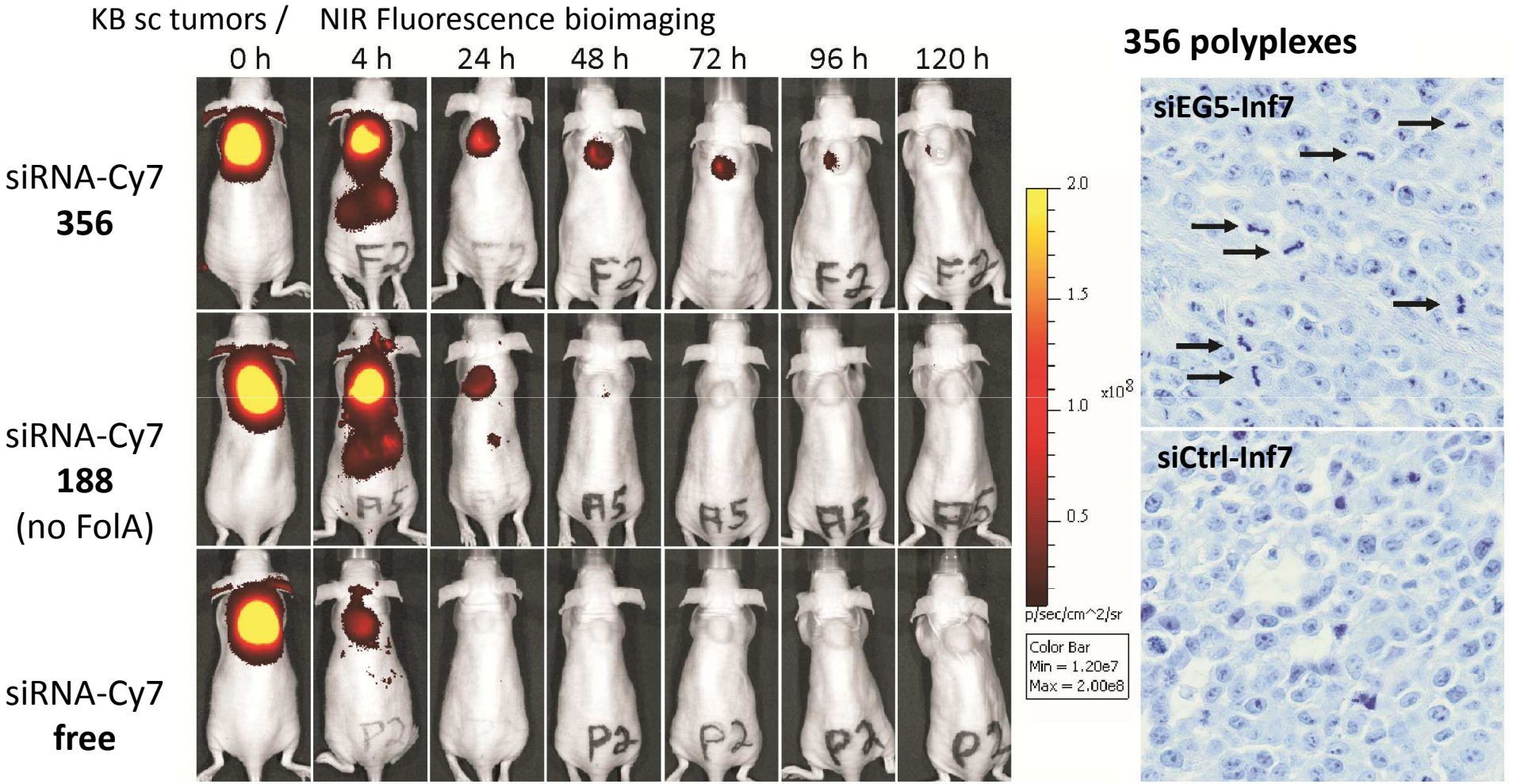
C. Dohmen *ACS Nano* 2012

Target gene silencing



Christian Dohmen, Laura Schreiner *ACS Nano* 2012

Folate dependent gene silencing in tumor



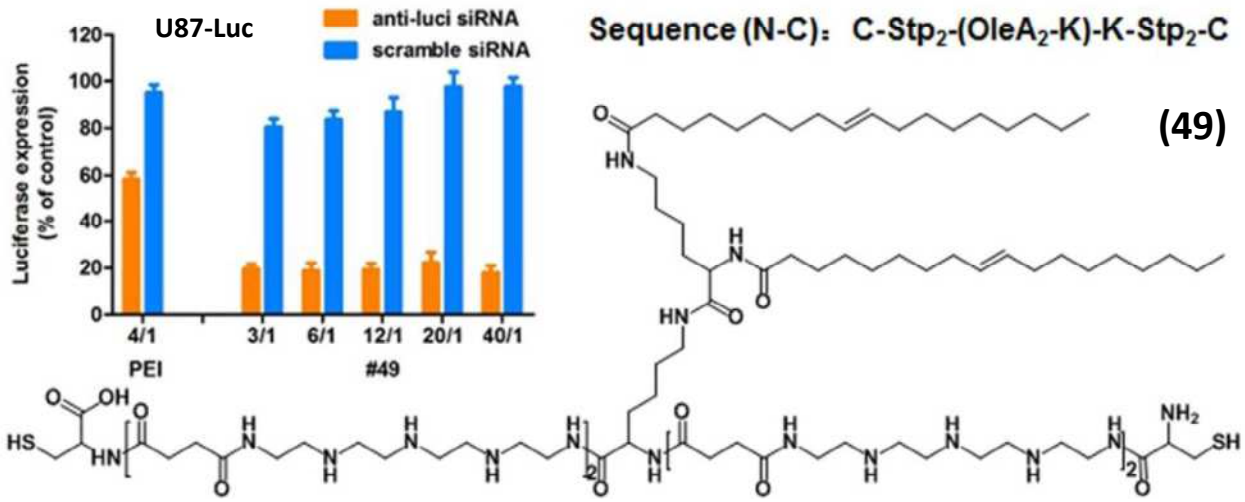
Intratumoral Cy7-siRNA Polyplexes

Aster formation only with siEG5-INF7/**356**

C. Dohmen *ACS Nano* 2012

Angiopep 2 for targeting:

Effective glioma-targeted siRNA delivery



Sequence (N-C): C-Stp$_2$-(OleA$_2$-K)-K-Stp$_2$-C

(49)

combination

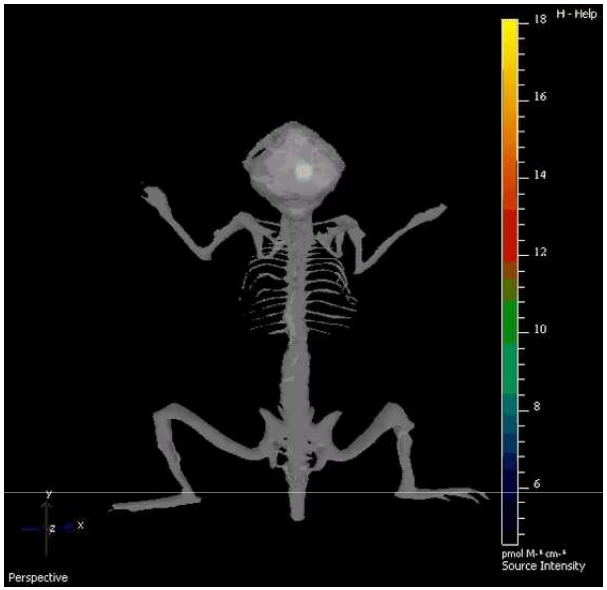
Sequence (N-C): [TFFYGGSRGKRNNFKTEEY]$_\alpha$-{[(C-Stp$_4$)$_2$]-$_{\alpha,\varepsilon}$K-PEG$_{28}$}$_\varepsilon$-K

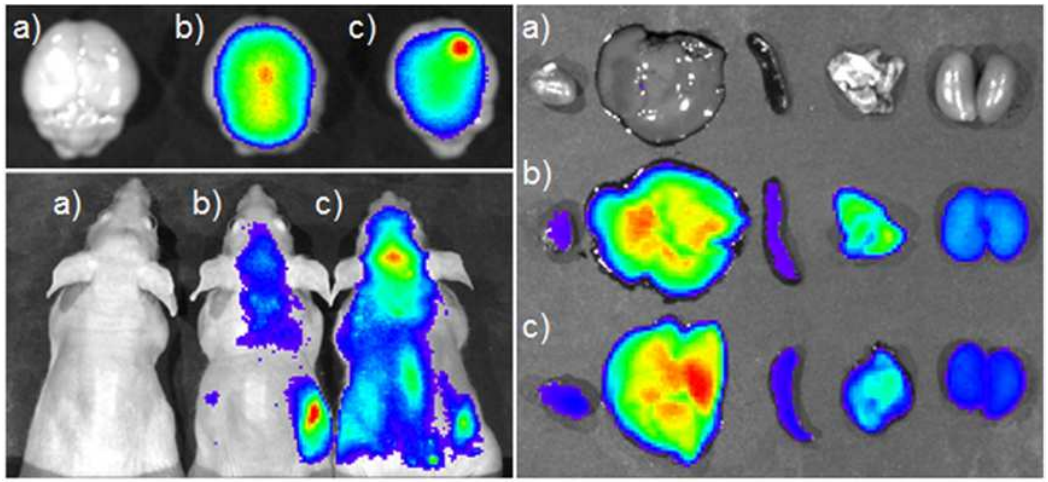
(727, targeting LRP)

Sai An, Dongsheng He, Ernst Wagner* & Chen Jiang* *Small* 2015

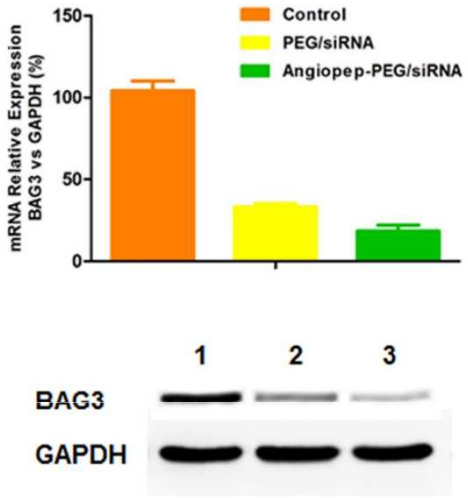
Angiopep 2 for targeting:

Effective glioma-targeted siBAG3 delivery

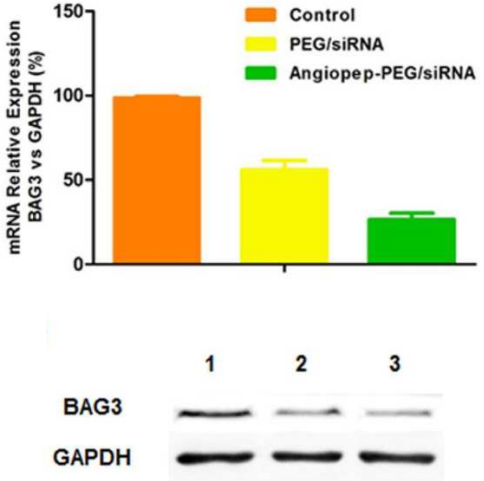




U87 *in vitro*

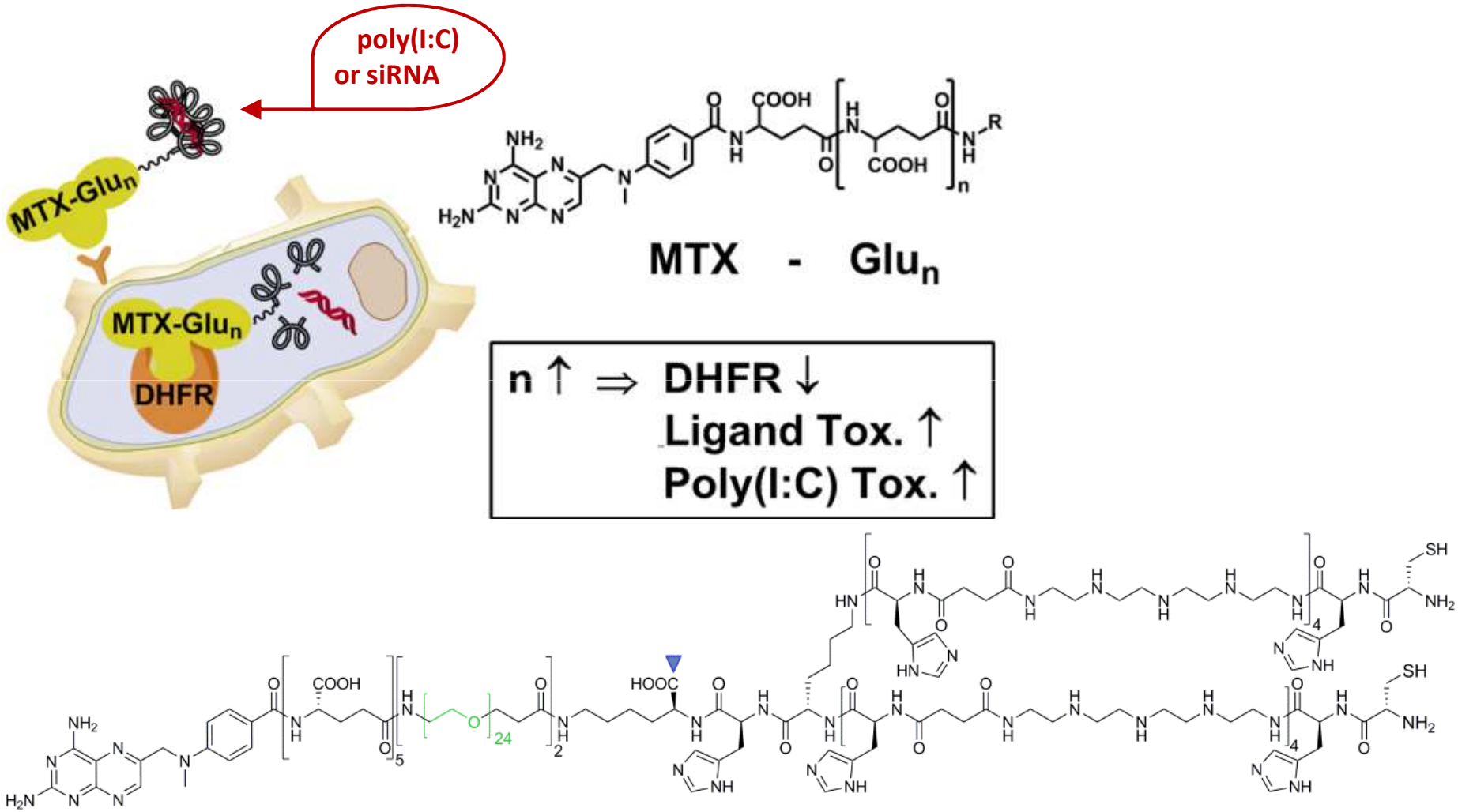


Glioma *in vivo*



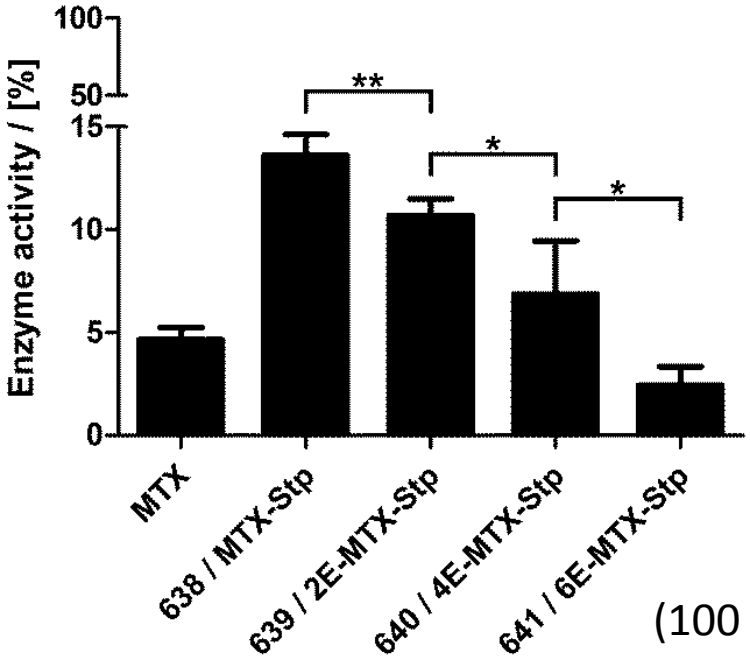
Sai An, *Small* 2015

Dual-functional polyglutamyl methotrexate ligands for potent combined tumoricidal activity

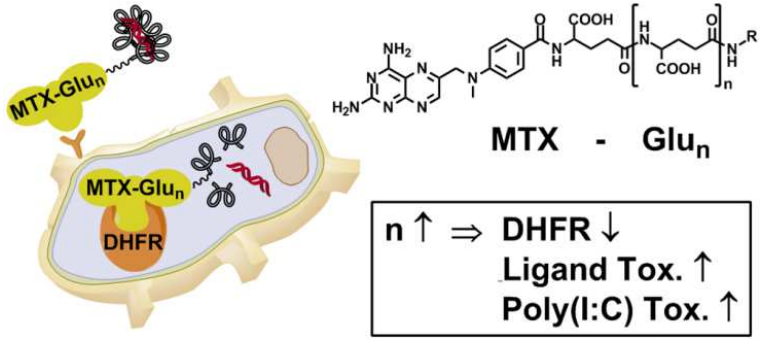


U. Lächelt, V. Wittmann, K. Müller et al *Mol Pharmaceutics* 2014

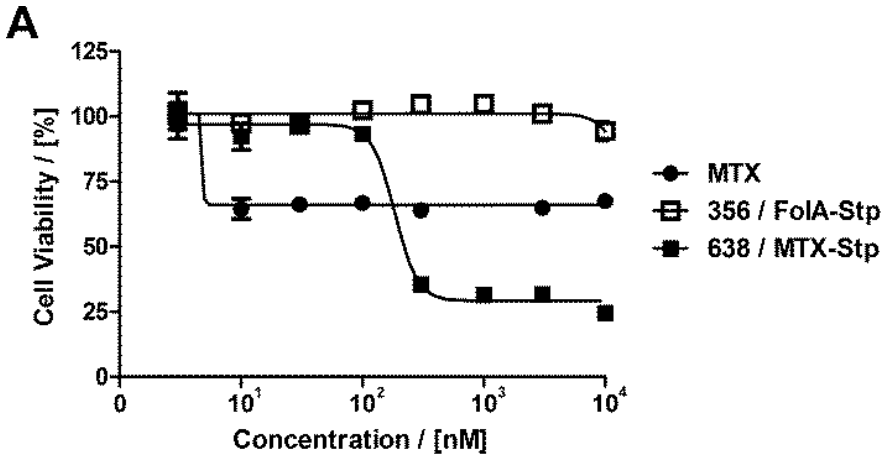
DHFR inhibition T e MTX oligomers)

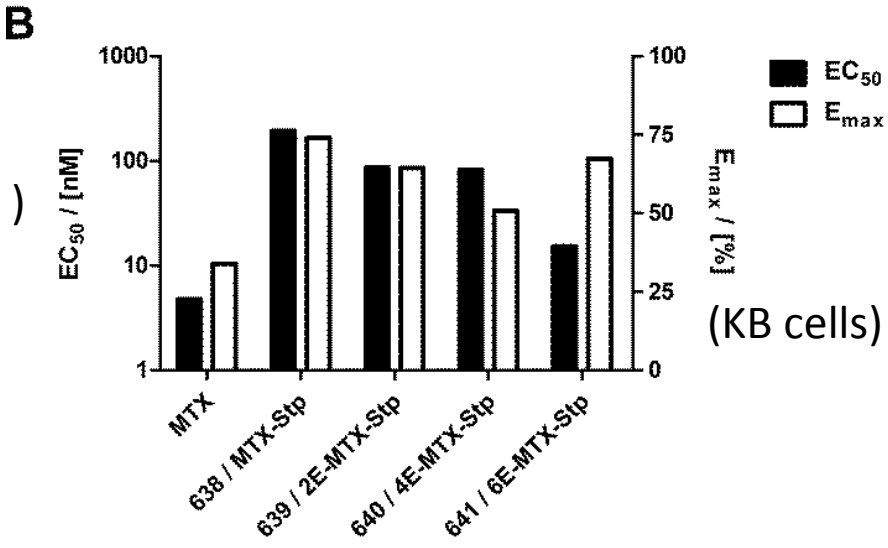


(100 nM MTX)



n ↑ ⇒ DHFR ↓
Ligand Tox. ↑
Poly(I:C) Tox. ↑

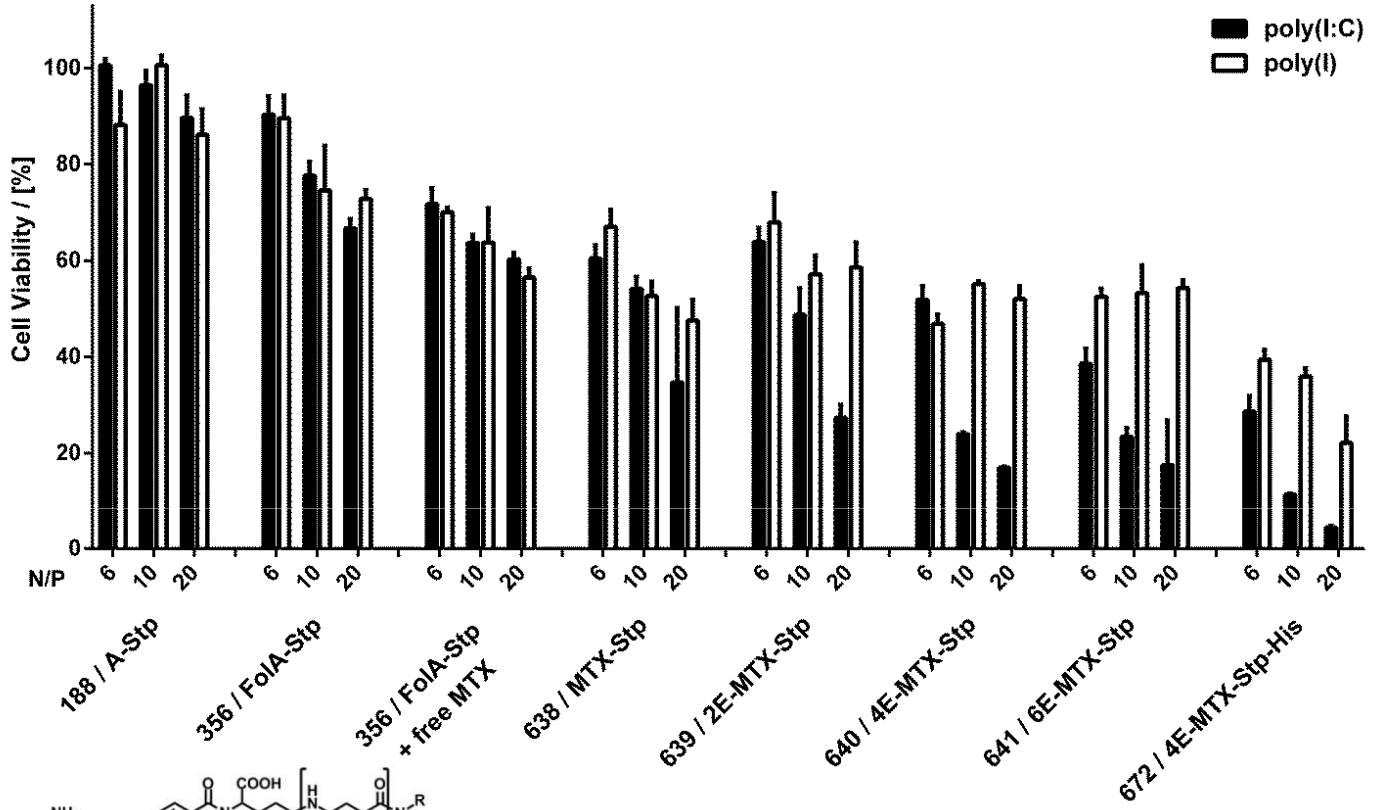


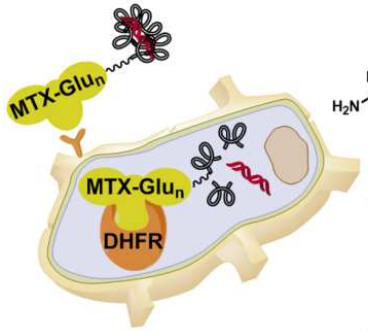


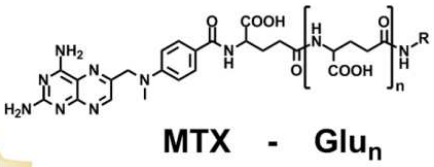
(KB cells)

U. Lächelt, V. Wittmann, K. Müller et al *Mol Pharmaceutics* 2014

Cell death by MTX / poly(I:C) nanoplexes







MTX - Glu$_n$

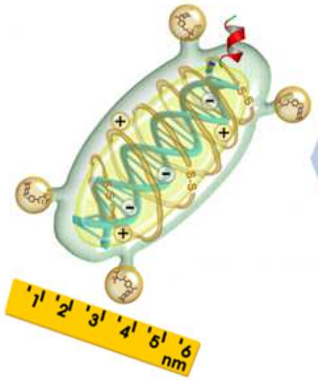
n ↑ ⇒ DHFR ↓
Ligand Tox. ↑
Poly(I:C) Tox. ↑

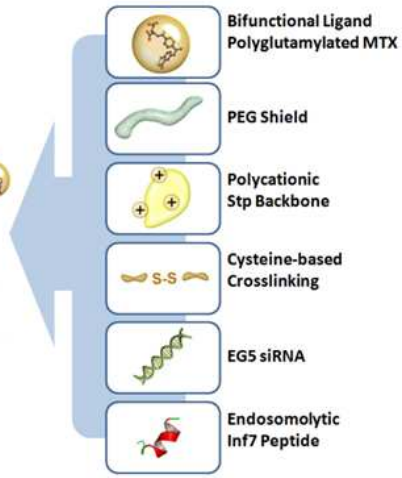
Poly(I:C): double stranded RNA, cytotoxic after delivery into cytosol

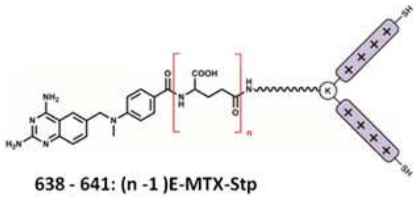
Shir et al 2006; Schaffert et al 2011

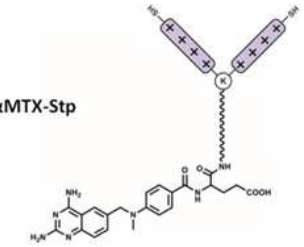
U. Lächelt, V. Wittmann, K. Müller et al *Mol Pharmaceutics* 2014

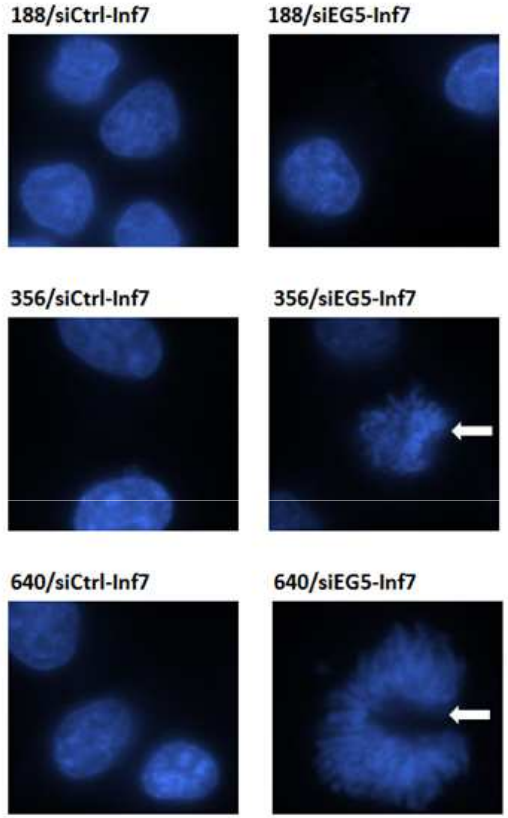
Gene silencing and cell death by 4E-MTX-Stp / siEG5 nanoplexes

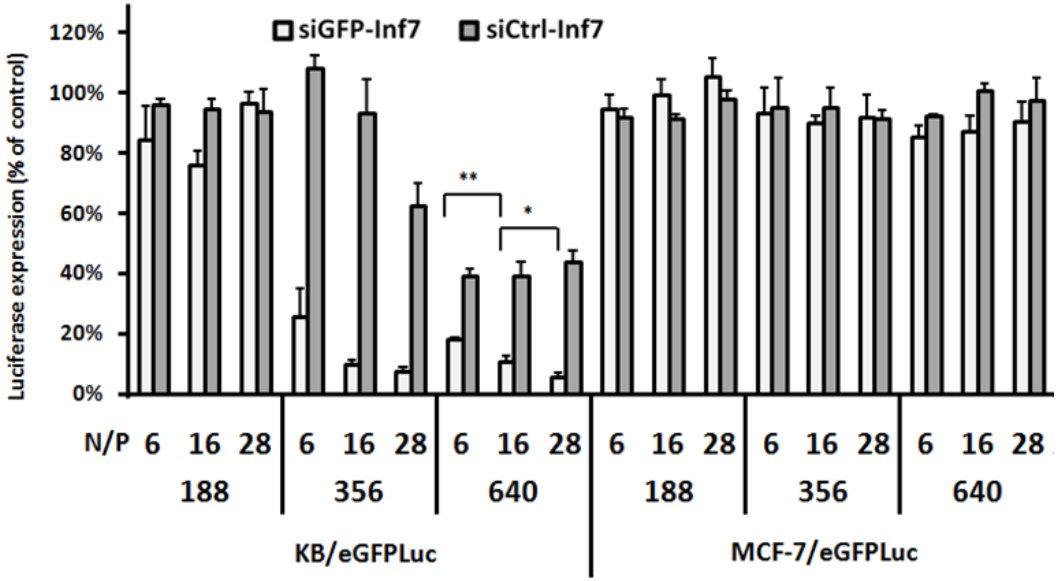








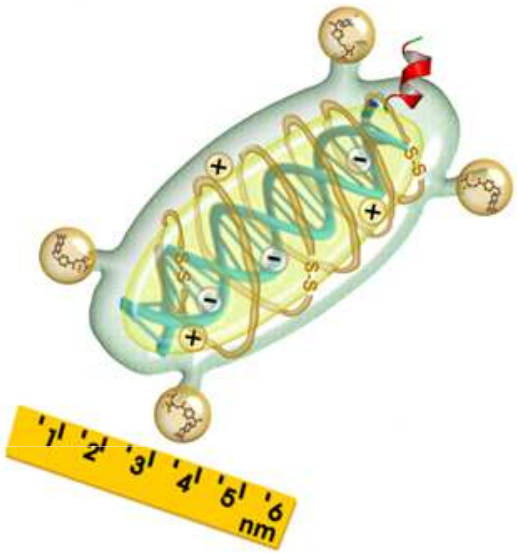


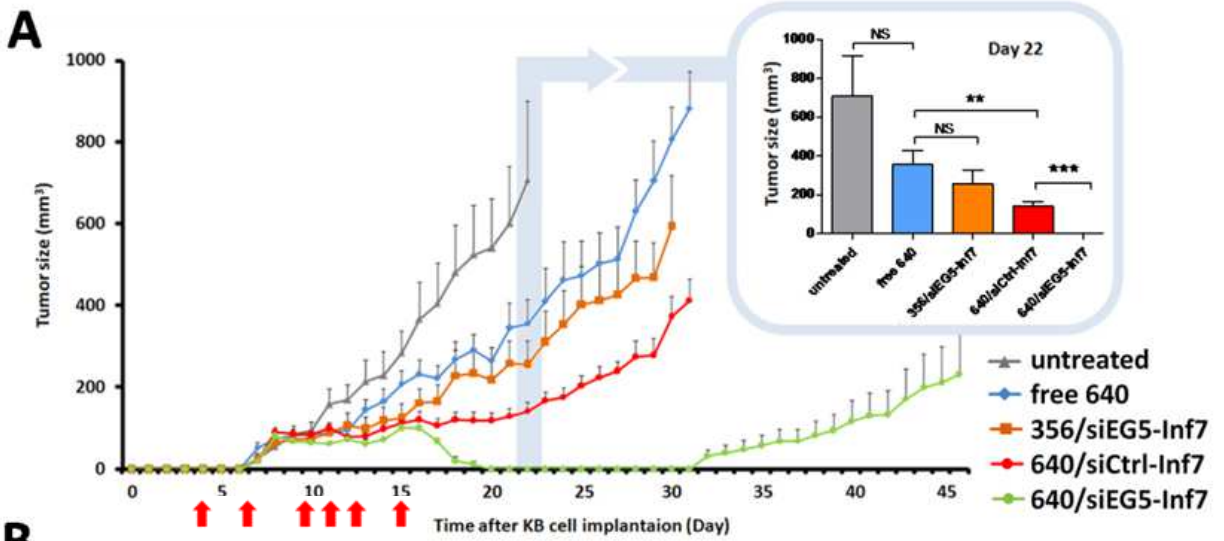


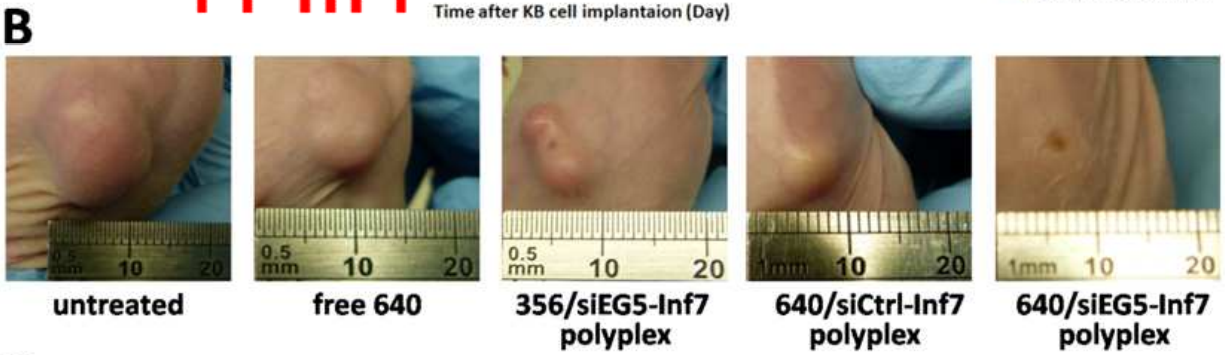
188 no targeting ligand
356: Folate
640: 4E-MTX

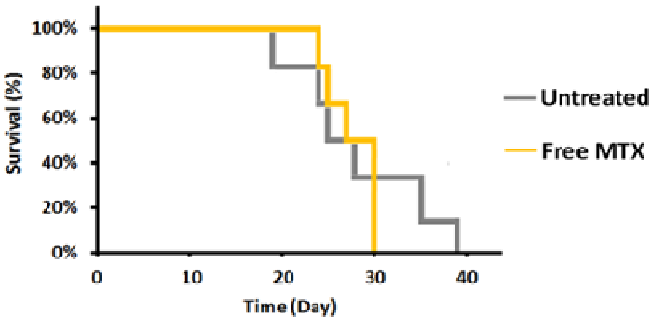
Dian-Jang Lee et al 2015 *submitted*

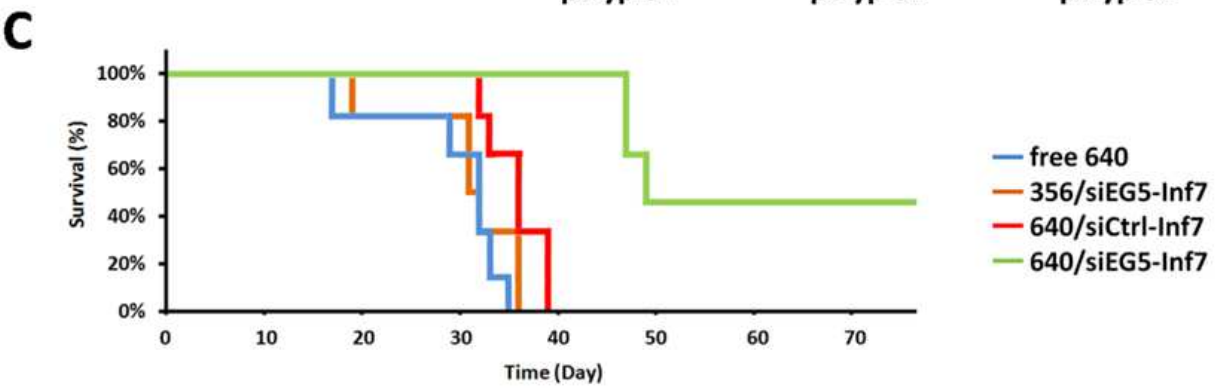
Cures by 4E-MTX-Stp / siEG5 nanoplexes











Dian-Jang Lee et al

Intracellular protein delivery by sequence-defined carriers

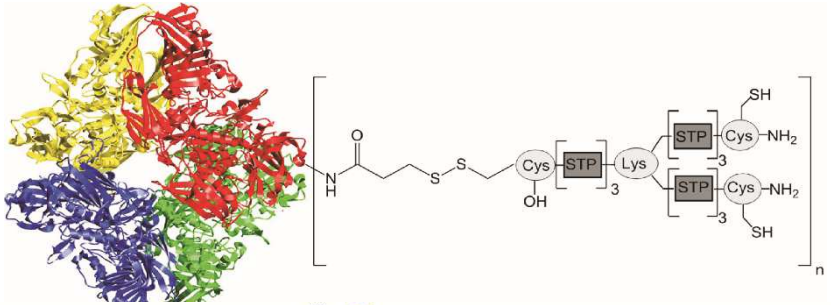


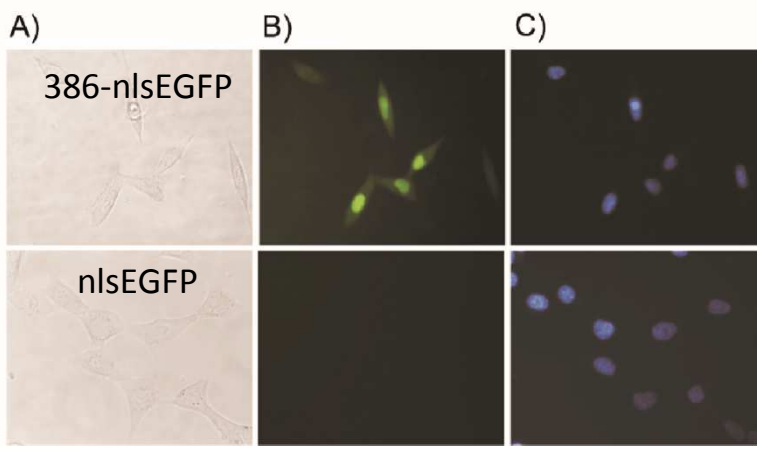

Kevin Maier, Irene Martin, Xiaowen Liu, Peng Zhang

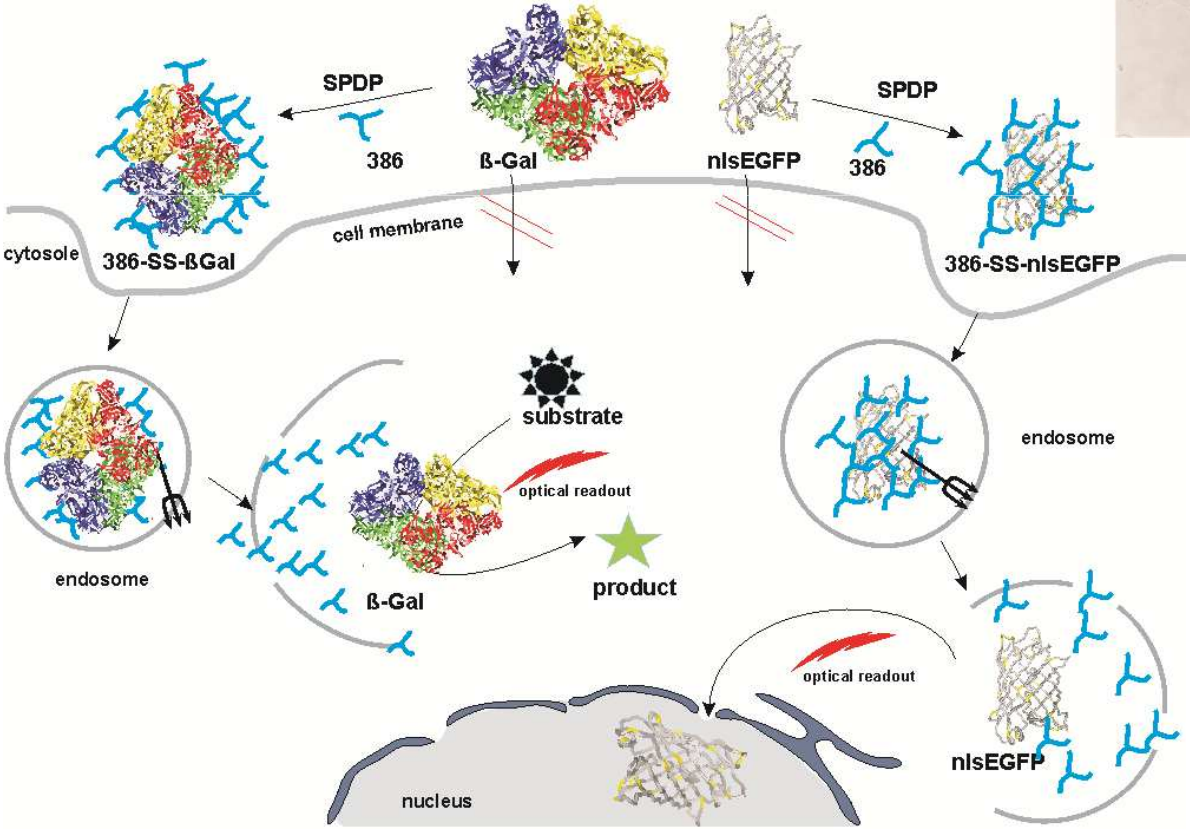
Three-arm shuttle for protein delivery

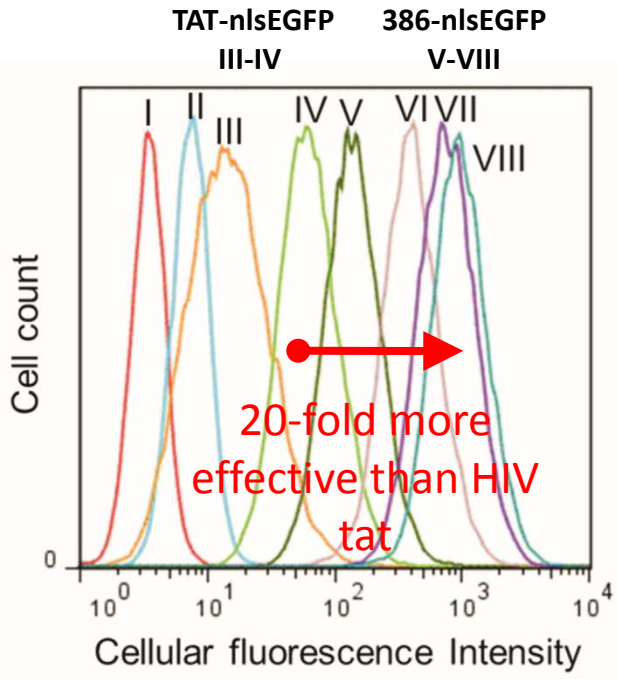
Reducible covalent disulfide linker

ß-Gal or nlsEGFP





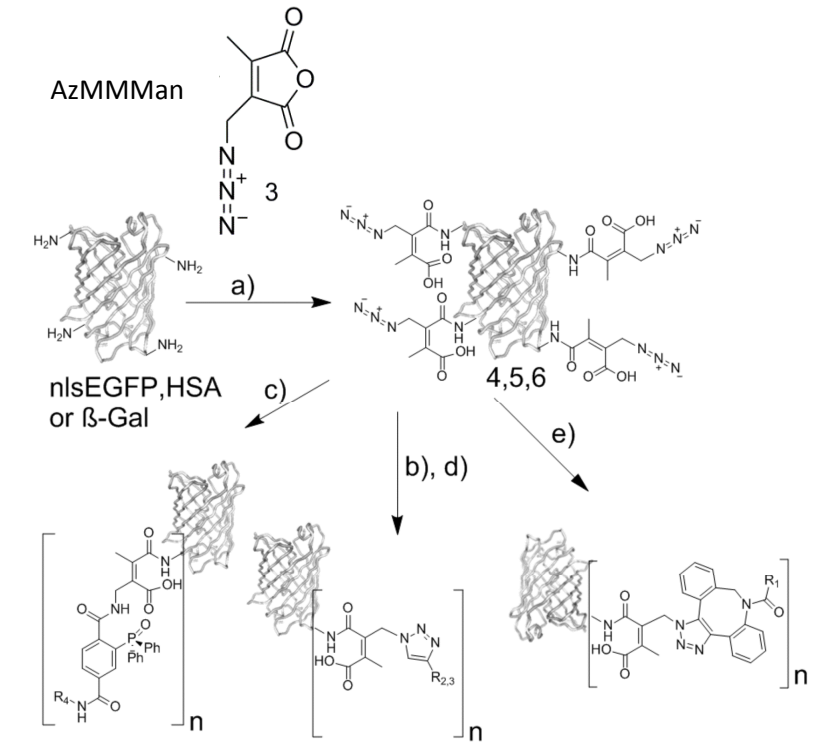


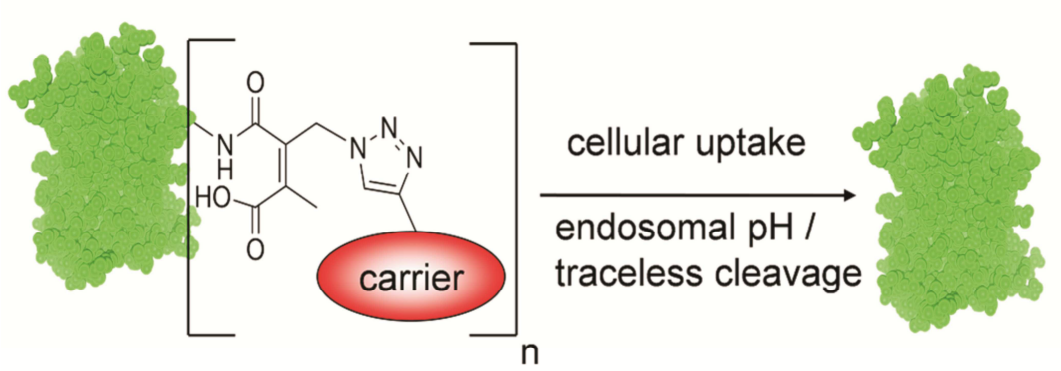


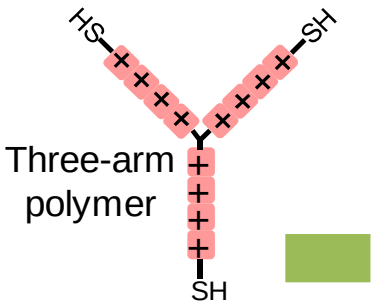
K. Maier, I. Martin *Mol Pharmaceutics* 2012

Three-arm shuttle for protein delivery

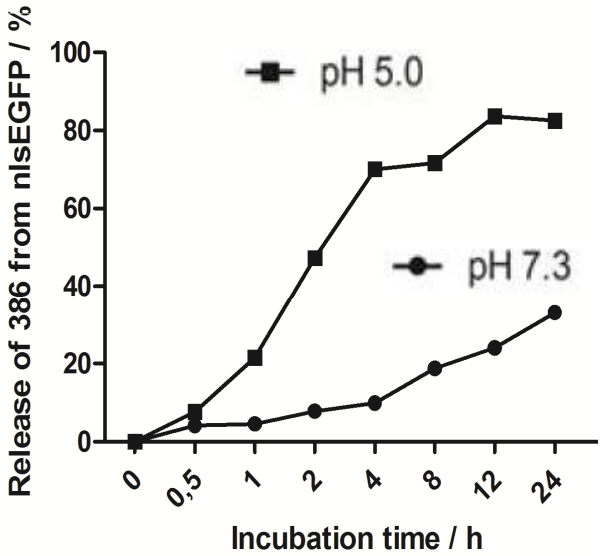
Acid-labile traceless click linker







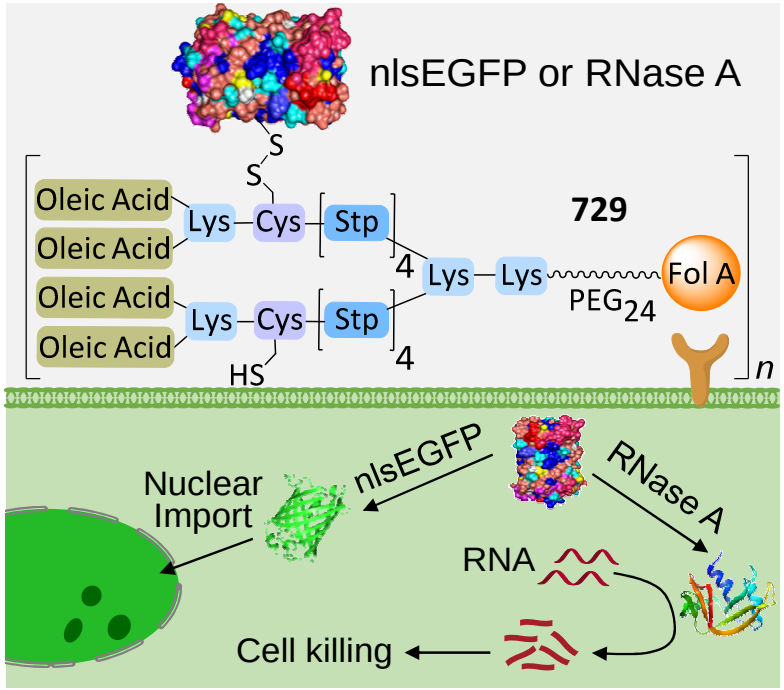
Carrier 386



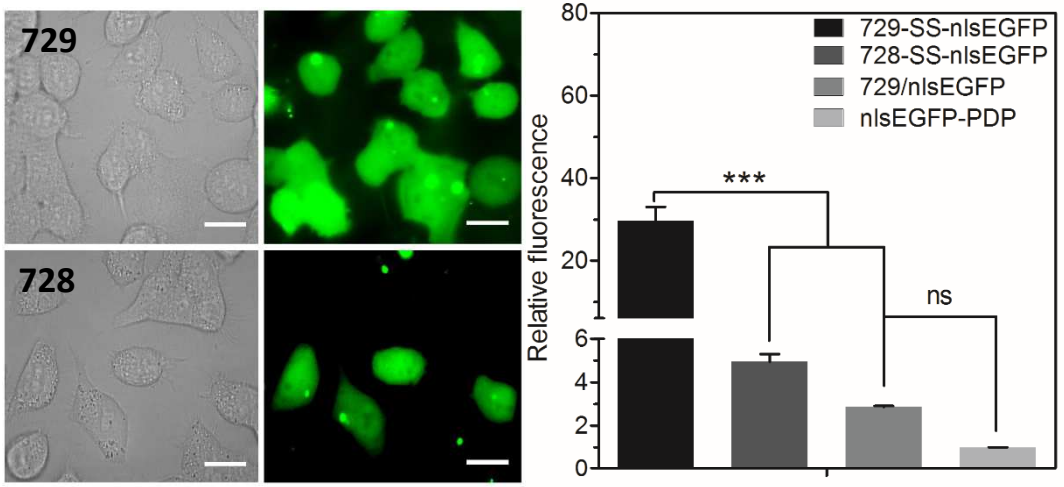
Kevin Maier *JACS 2012*

FolA targeted shuttle for RNase A delivery

Oligomer library screen (reducible disulfide linker)

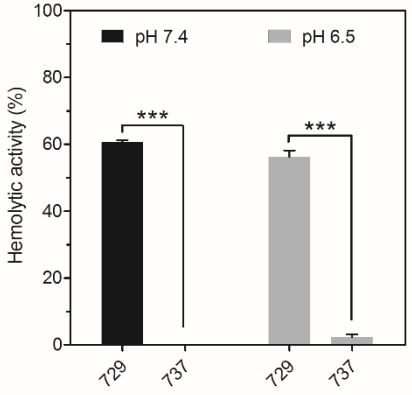


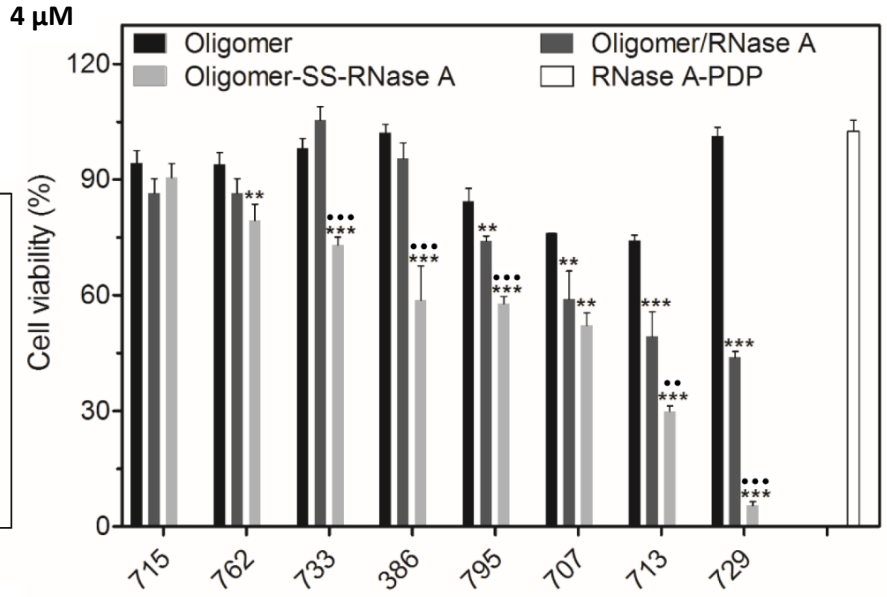
After 48 hours !



nlsEGFP conjugate	Z-average (nm)	zeta (mV)
728	29.7 ± 0.5	13.1 ± 0.7
729	36.6 ± 1.0	15.0 ± 0.7

Worm-like rods, ~10 nm diameter, resembling
PEG-coated H_I lipid micelle rods





Peng Zhang et al *Adv Funct Mater* 2015

Pharmaceutical Biotechnology (Ernst Wagner)

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