Brain Targeted Nanomedicine: More Than Just Crossing the BBB

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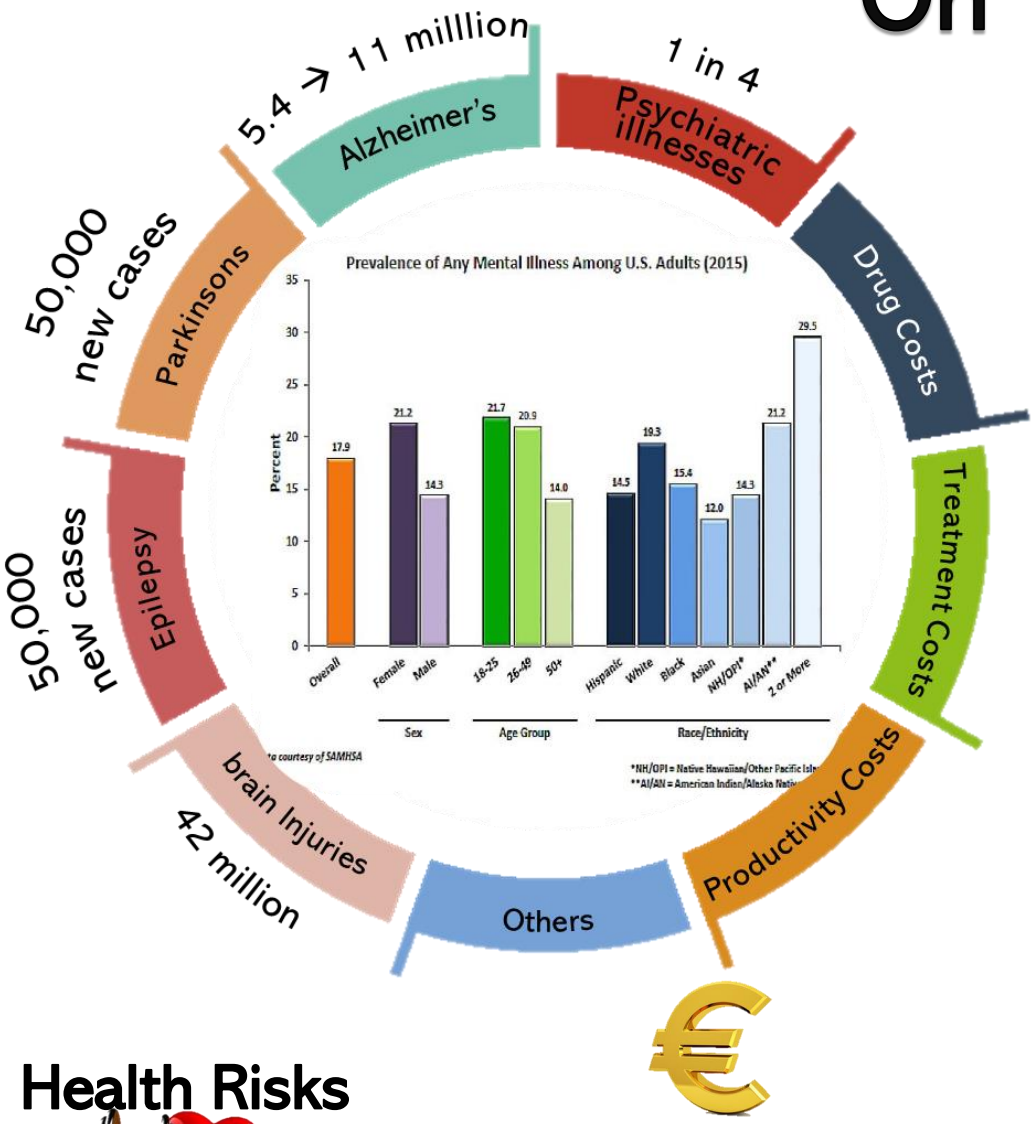
M.Sc. Maria Vittoria Grazioli

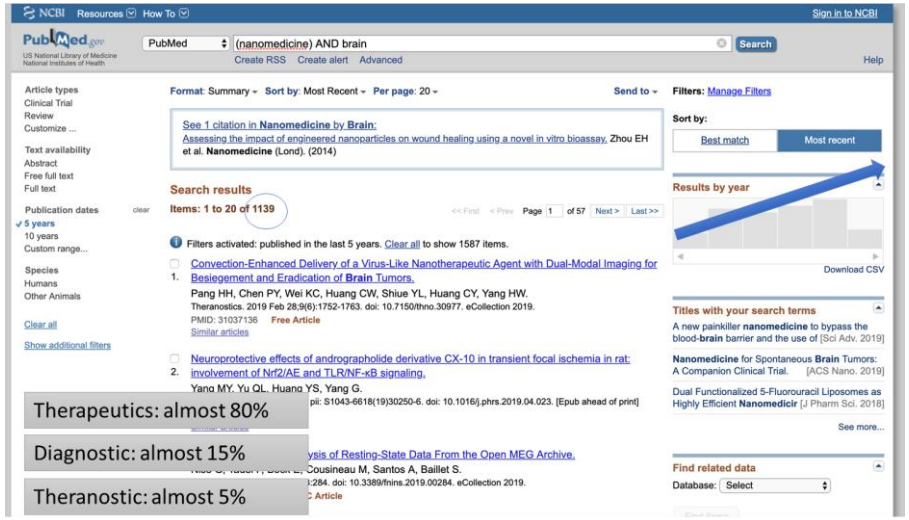
Prof. Giovanni Tosi

Prof. Barbara Ruozi

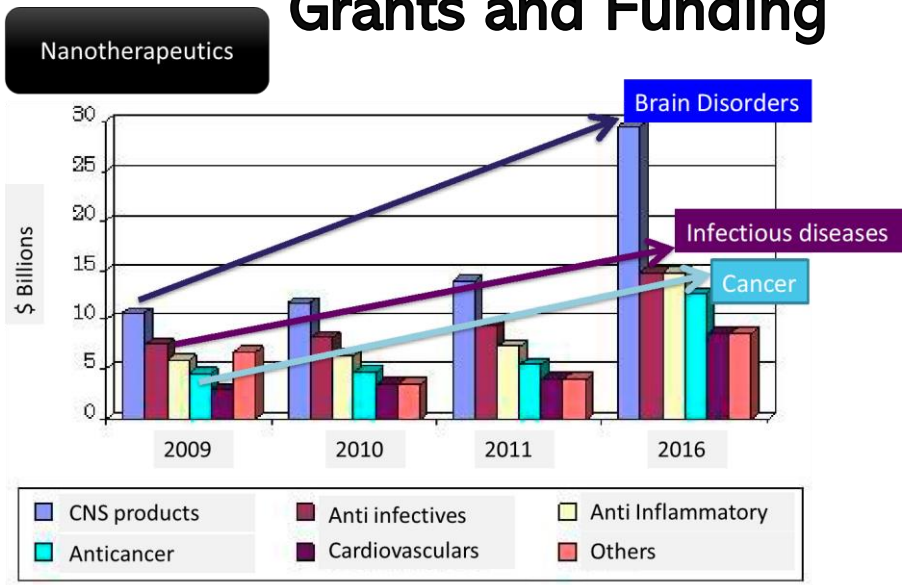
On The Rise!!!



Literature



Grants and Funding



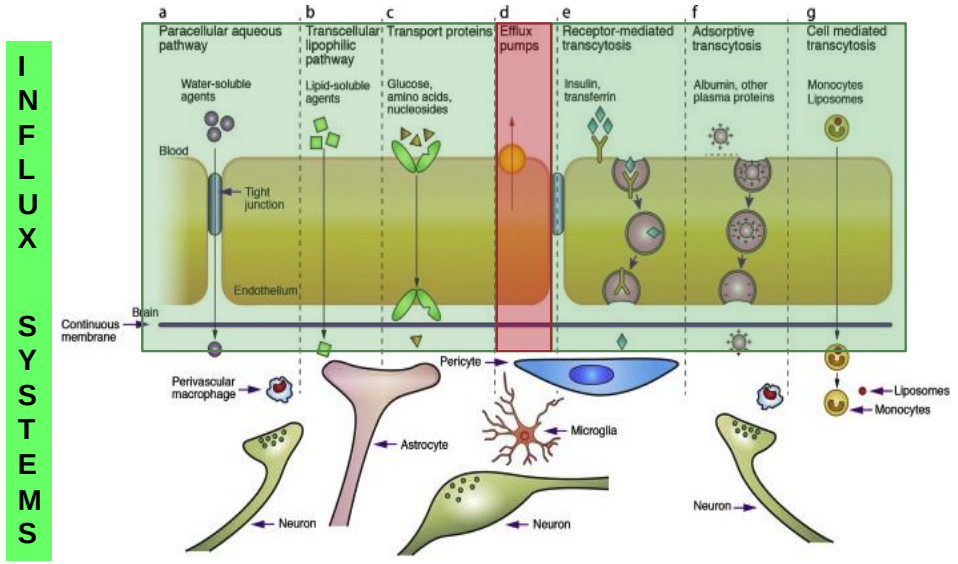
Efforts in Nanomedicine application 2009-2016

Health Risks

€798 billion/year
(2010) 35%

What we know:

- The barriers are protecting environments
- The Blood Brain Barrier (BBB) protects the life of our brain
- **BBB blocks 80% of drug transport**



Barriers

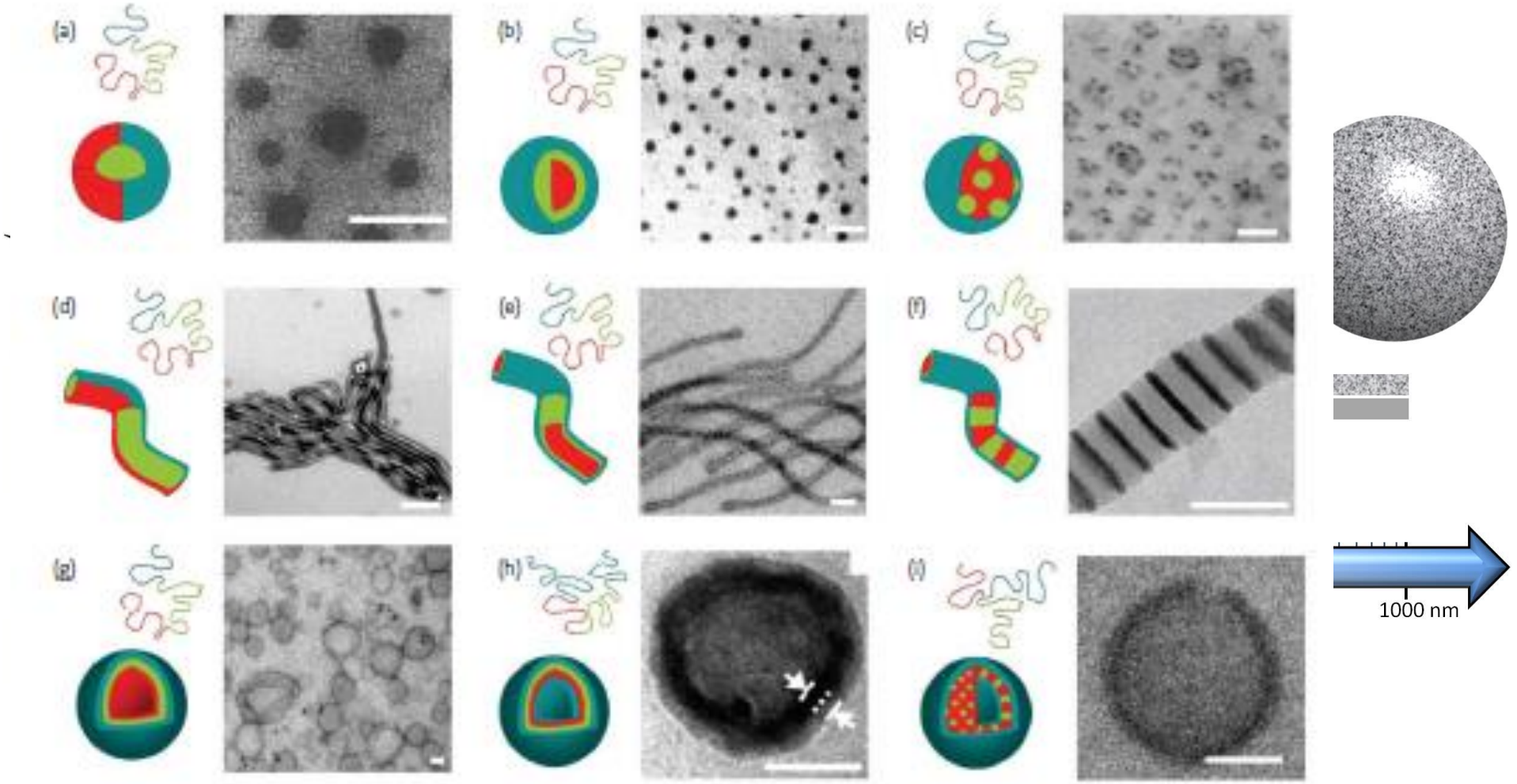
Diseased BBB

BBB state in diseases could be:

- Healthy-like #wehaveaproblem
- Almost ILL mild damage
- Completely destroyed severe damage

- **Target Blood Brain Barrier**
- **Target specific cells or receptors**
- **Target pathogens**

Overcoming barriers



DOI: 10.1007/s11095-014-1545-6 http://dx.doi.org/10.1016/S1748-0132(08)70043-4

Vectors

Marketability

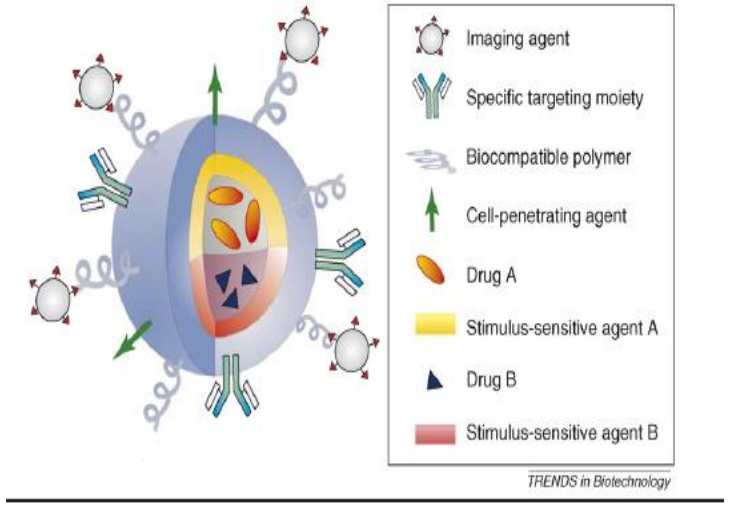
Developing nanomedicines

Pharma Companies:
large amounts
GMP conditions
high yield
low cost

Simpler is better

In the case of brain diseases, we are almost forced to introduce surface engineering and produce multifunctional targeted nanomedicines

Surface Engineering



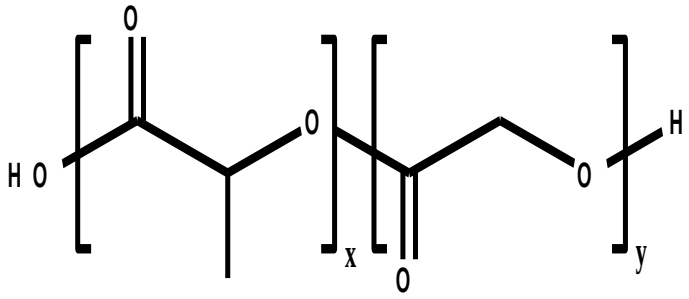
***Good* ligands for *Good* receptors:**
Overexpressed at BBB
Selectivity (non ubiquitous)
High Binding

Take into account:
Possible saturation and size restriction
Mechanism of BBB crossing should be endocytosis/transcytosis/pinocytosis

Production

PLGA nanoparticles

PLGA: poly(lactic-co-glycolic acid)



- Safe (FDA approved)
- Not extremely expensive
- Possible to chemically modify with ligands
- Double emulsion nanoparticles
  - 200 nm
  - -20 mv charge
  - Possible to entrap various molecules

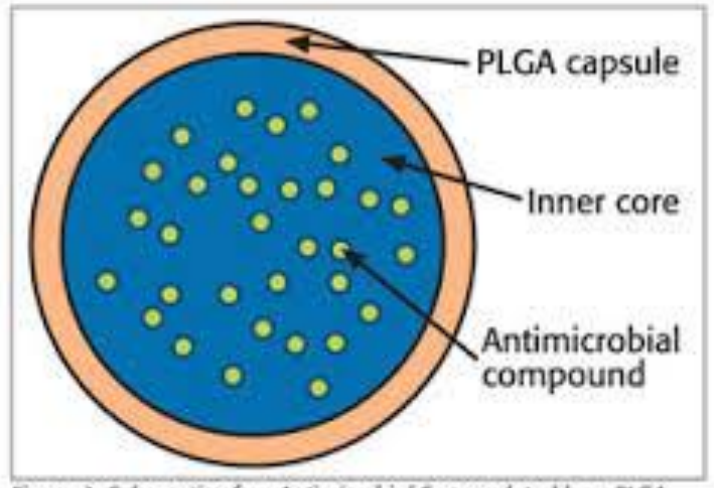
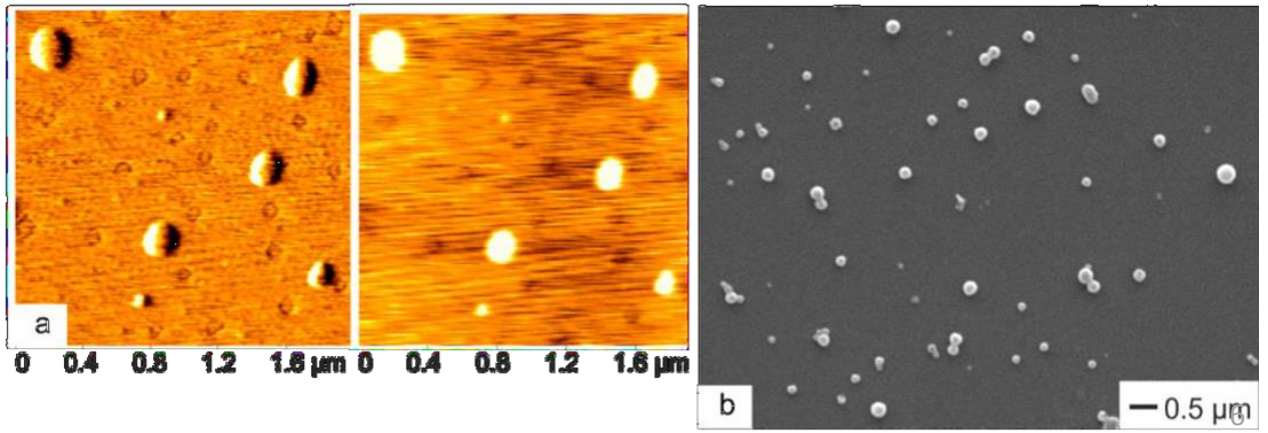


Figure 1: Schematic of an Antimicrobial Encapsulated by a PLGA Nanoparticle



Nanomedicine (NPs+PROTEIN): ACCUMULATION in NEURONS

MODEL PROTEIN DELIVERY

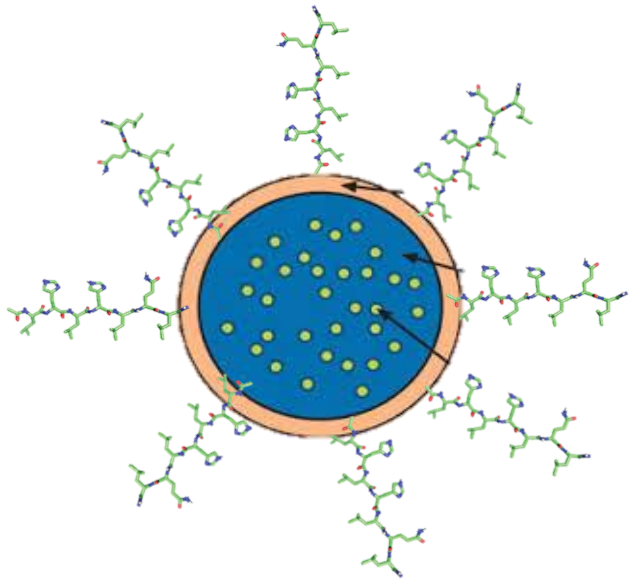
CONFOCAL: HURLER MICE MPSI

PLOS ONE | PlosOne, 2016

RESEARCH ARTICLE

Targeted Polymeric Nanoparticles for Brain Delivery of High Molecular Weight Molecules in Lysosomal Storage Disorders

Marika Salvalaio[1,4☯], Laura Rigon[1☯], Daniela Belletti[2], Francesca D'Avanzo[1,6], Francesca Pederzoli[2,4], Barbara Ruozi[2], Oriano Marin[3,5], Maria Angela Vandelli[2], Flavio Forni[2], Maurizio Scarpa[1,6], Rosella Tomanin[1]*, Giovanni Tosi[2]*



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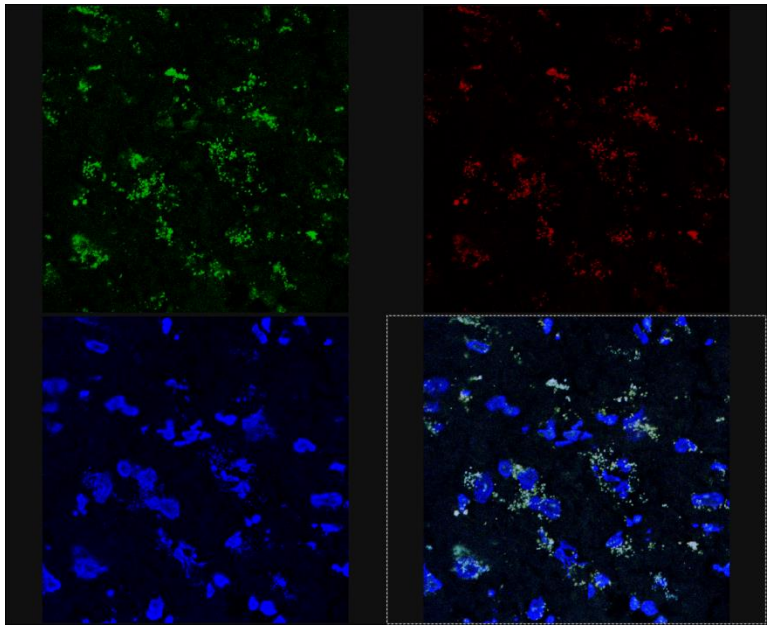


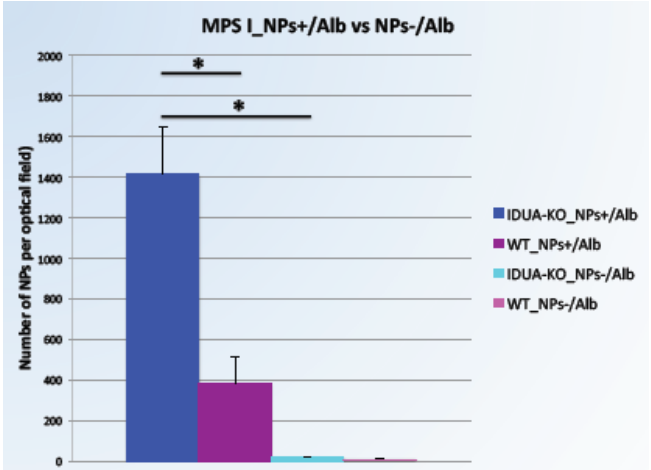
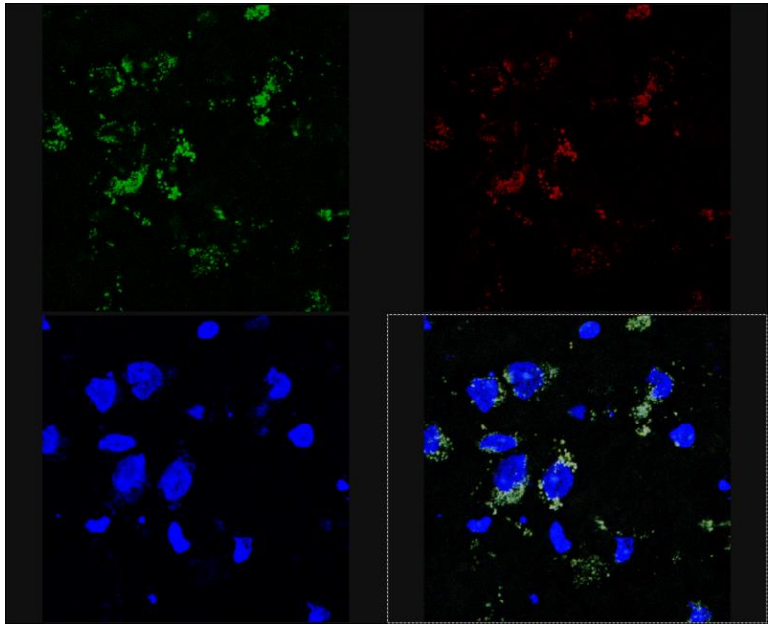


Fig.4: Number of NPs per optical field evaluated on brain slices by confocal microscopy. Hurler and wild-type mice (both *n=3*) were injected with NPs+/Alb (left) and NPs-/Alb (right). * *p<0.05*

HURLER MICE MPS_II



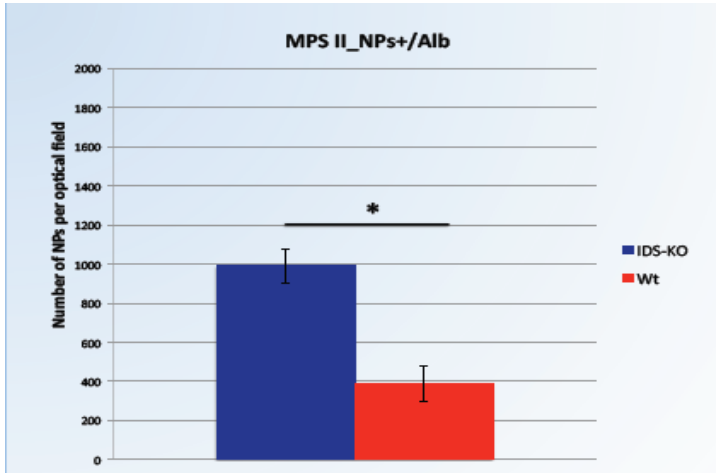
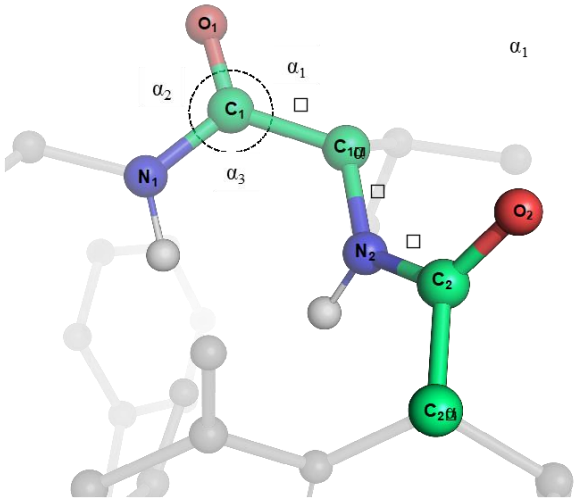
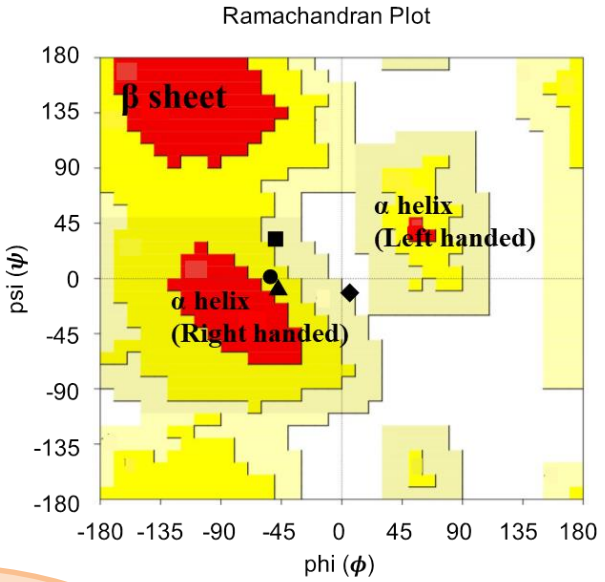
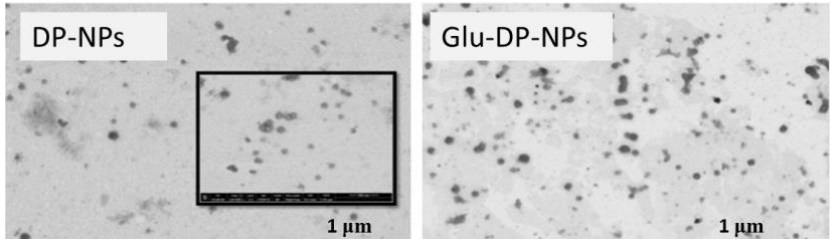


Fig.5: Number of NPs per optical field evaluated on brain slices by confocal microscopy. Hunter and wild-type mice (both *n=3*) were injected with NPs+/Alb.
* *p<0.05*



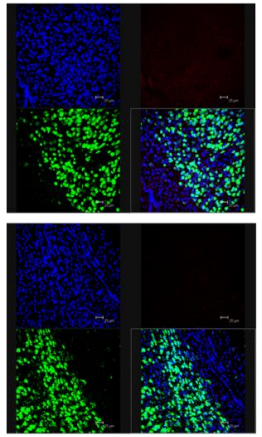


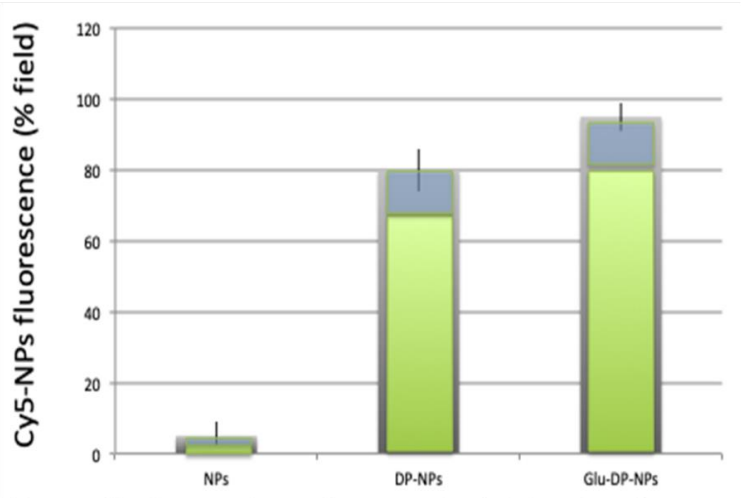
Sample name	Size (SD)	PDI	Zeta Potential (SD)
NPs	225 (12)	0.27 (0.01)	-20 (5)
DP-NPs	249 (10)	0.18 (0.03)	-34 (4)
Glu-DP-NPs	259 (21)	0.23 (0.02)	-29 (5)



TARGETING

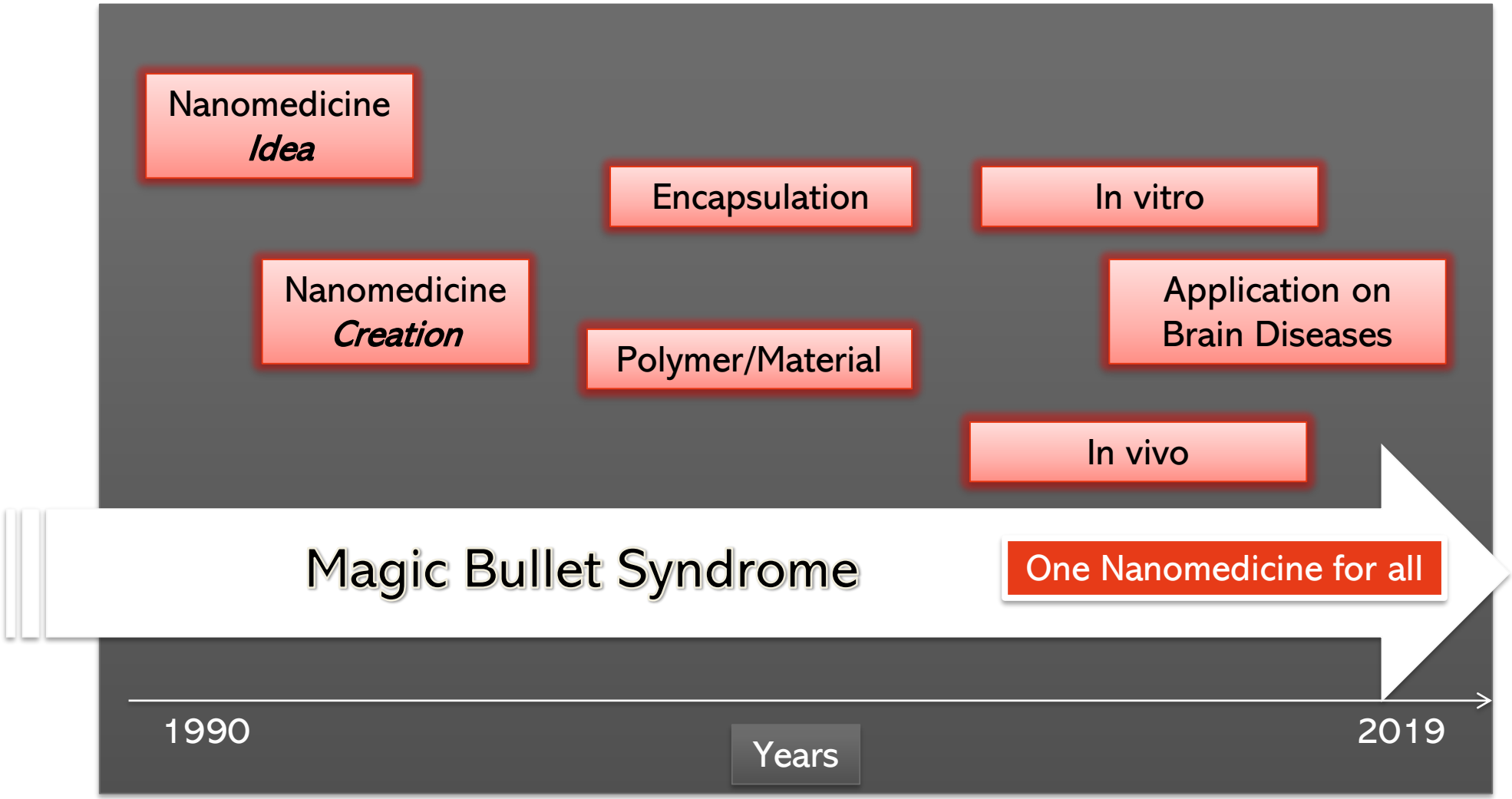
- G7
- BBB Deltorphan opiod peptide
  - Derivatives
- Viral coat peptides
- Antibodies
  - BBB
  - Plaques
  - GBM specific
- PEG



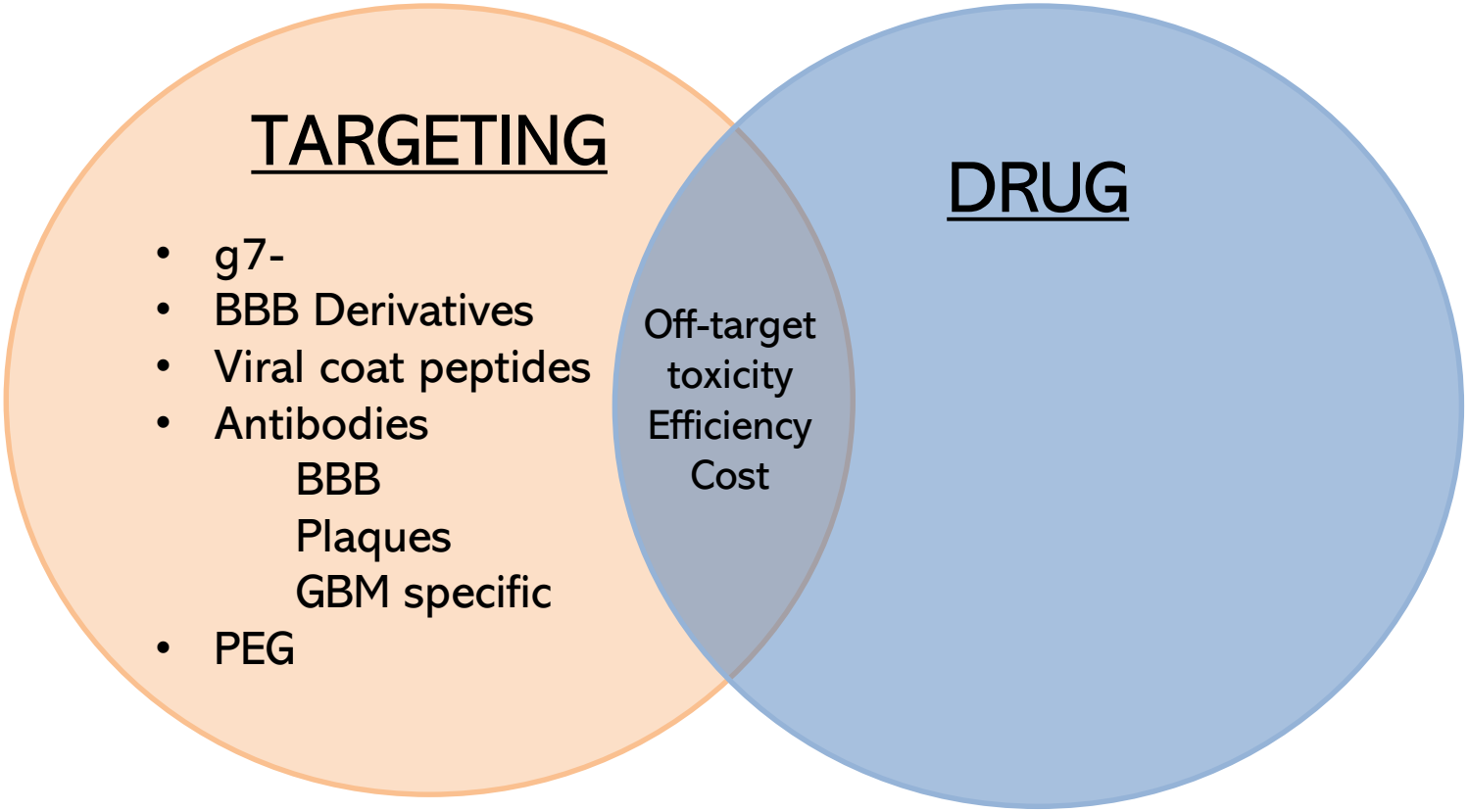


F) Representative confocal image of brain parenchyma after systemic administration of DP-NPs. Nucleus are stained with DAPI (blue signals), Neurons are stained with NEUN (green signals) and DP-NPs are tagged with Cy5 (red signals) anda semiquantitative analysis of CY5-NPs signals % per field. Grey bars indicated the total red signals meaning labelled NPs, green bars indicated the percentage of red signals co-localizing with DAPI and NEUN positive signals; blue bars represent the percentage of red signals co-localizing with DAPI signals

Targeting NOT Limited to Targeting Ligands!

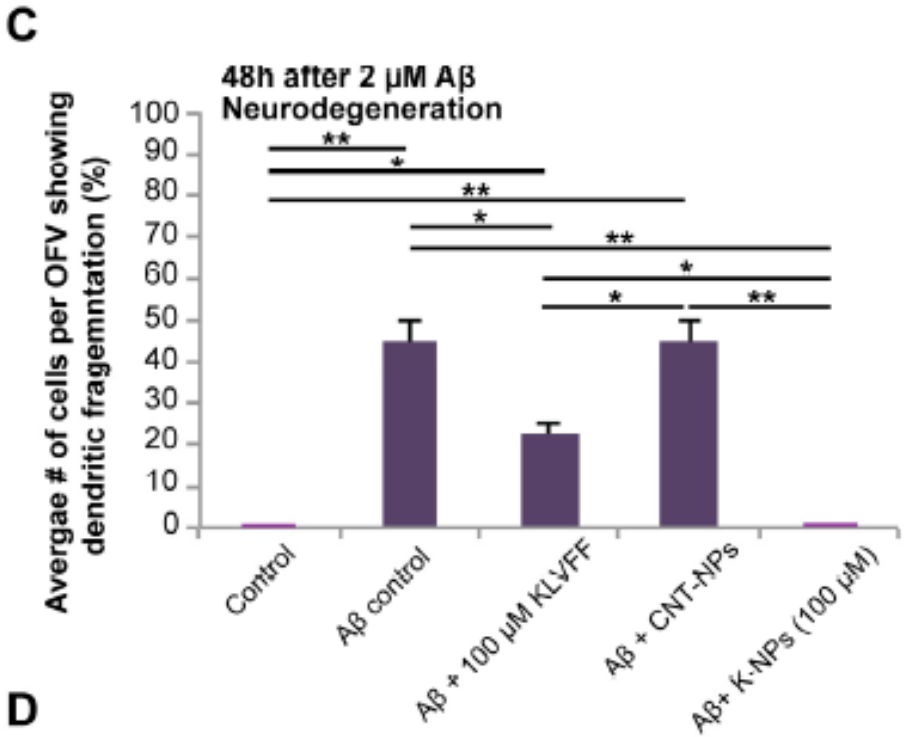


Increasing Drug Potential

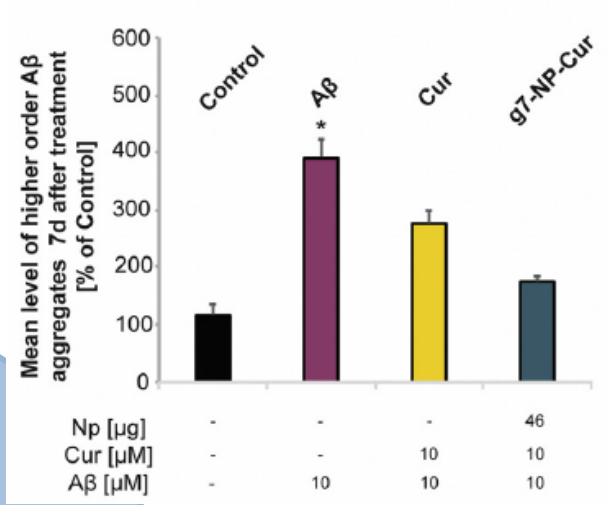


Anti amyloid β molecules to combat Alzheimer's Disease

KLVFF

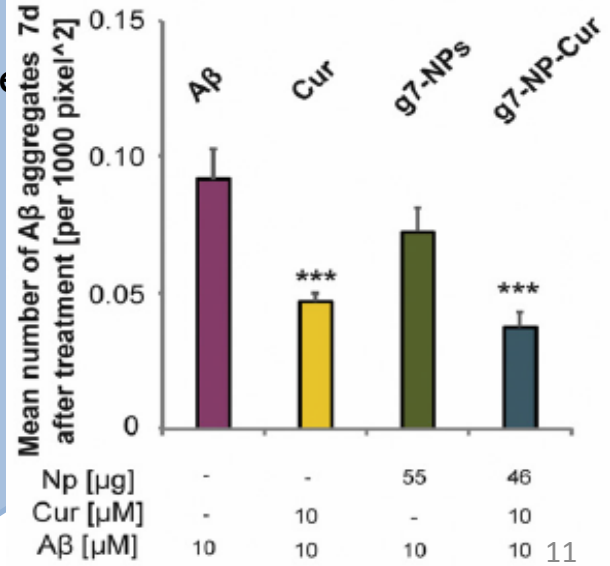


Curcumin



DRUG

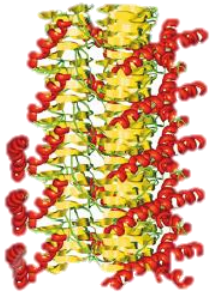
KVLFF peptide - Alzheime
Curcumin



Prion Disease Treatment

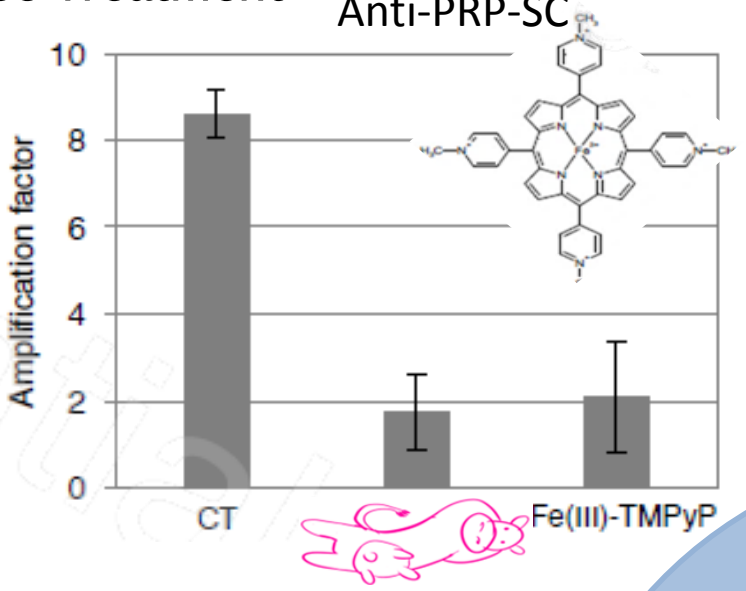
PRP-C

PRP-SC

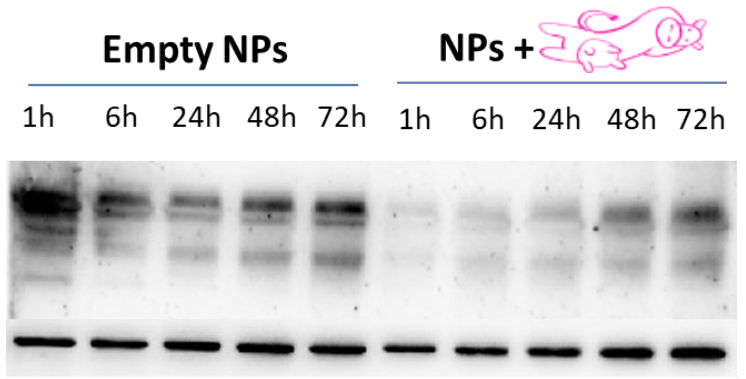


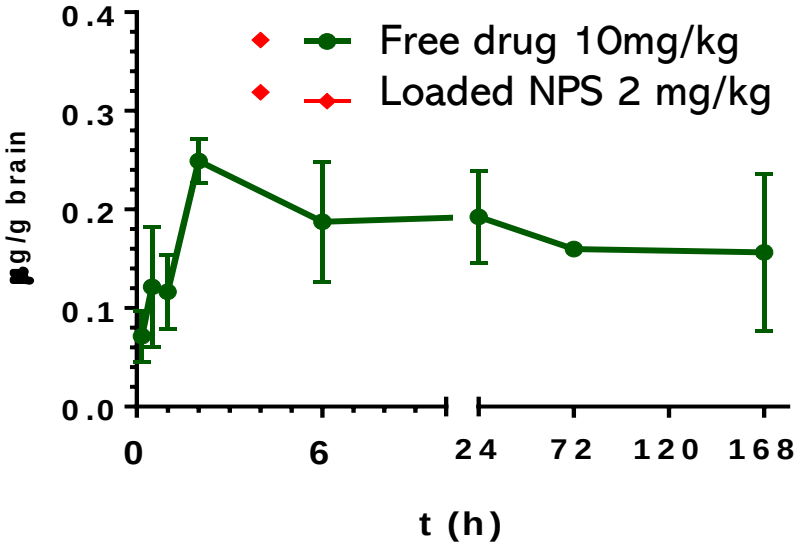
Granular neurons (5uM)

Anti-PRP-SC



Anti-PRP-C





DRUG

KVLFF peptide - Alzheimer's
Curcumin
Anti Prion Diseases

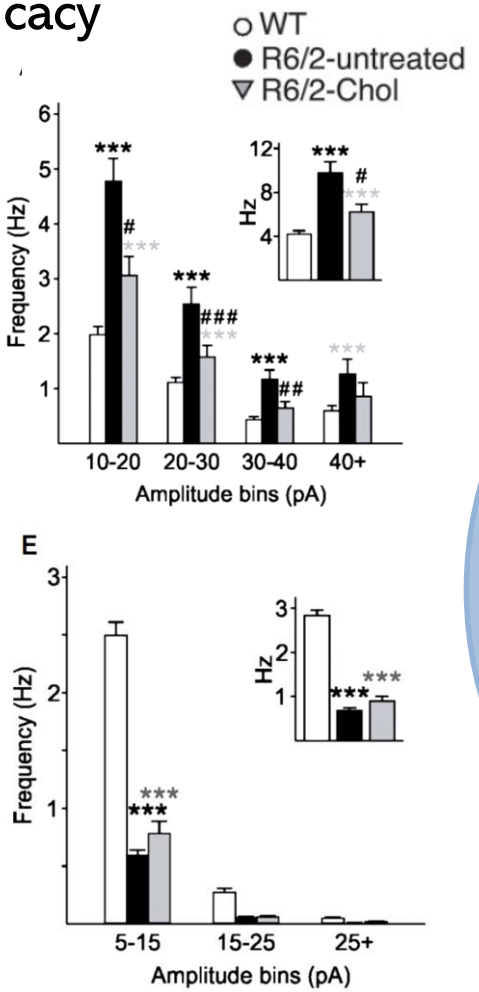
Cholesterol for Huntington's Disease

In vivo efficacy

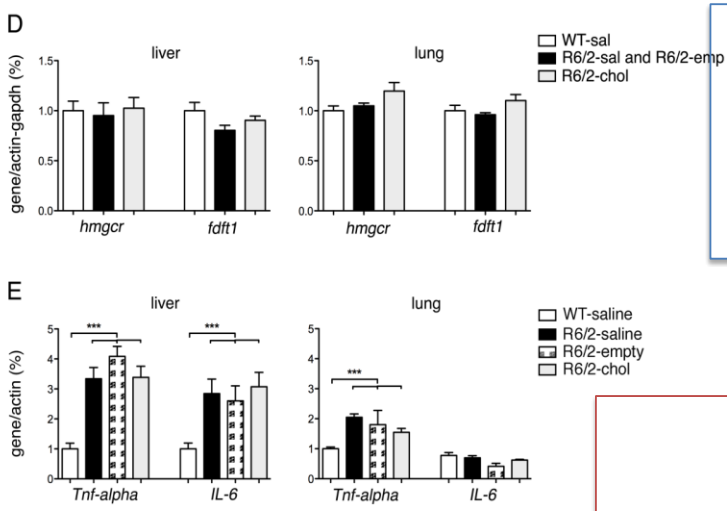
Gabaergic transmission

Systemic injections of chol-g7-NPs rescue synaptic alteration in HD R6/2 mice.

RESCUE



In vivo safety

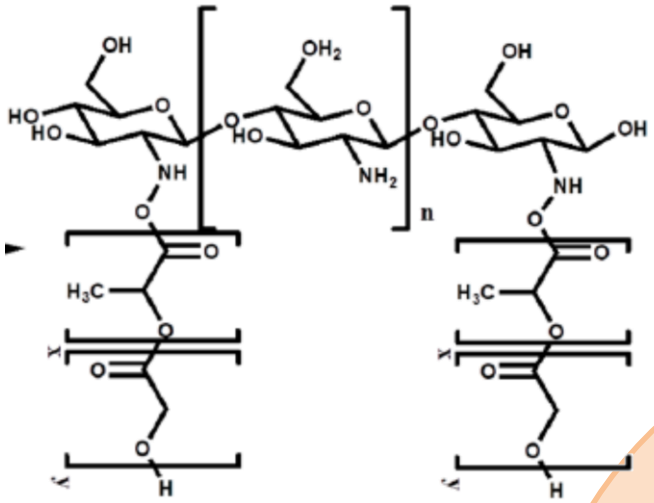


polymers

MDPI

Article

Investigating Novel Syntheses of a Series of Unique Hybrid PLGA-Chitosan Polymers for Potential Therapeutic Delivery Applications



NANOFORMULATION

Core component:
PLGA conjugates
PLGA hybrid materials
Hybrid nanoparticles
Lipids
cholesterol

Method:
Emulsion (single/double)
Nanoprecipitation,
Salting out

Solvents – Stabilizers - Stresses
(sonication/heating/purification)

Biocompatibility
Protein Corona
Toxicity

Encapsulation %
Stability
Release

TARGETING

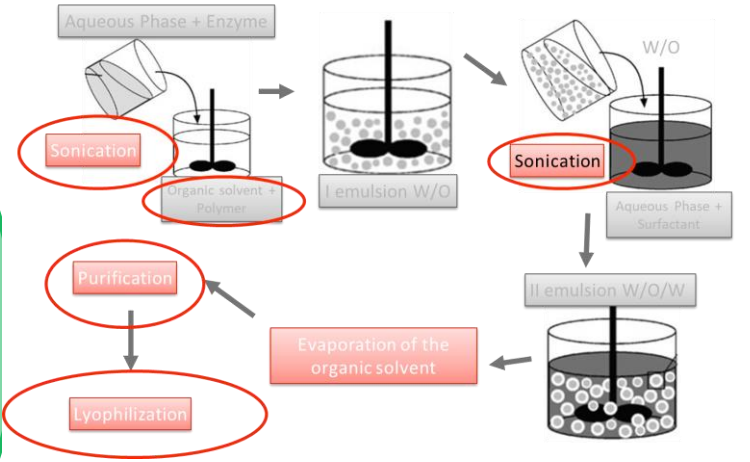
G7 – BBB (and variants)
A6 - Glioblastoma
PEG - First pass clearance
antibodies.
Other opioid peptides

Protection
Immune
response
Toxicity

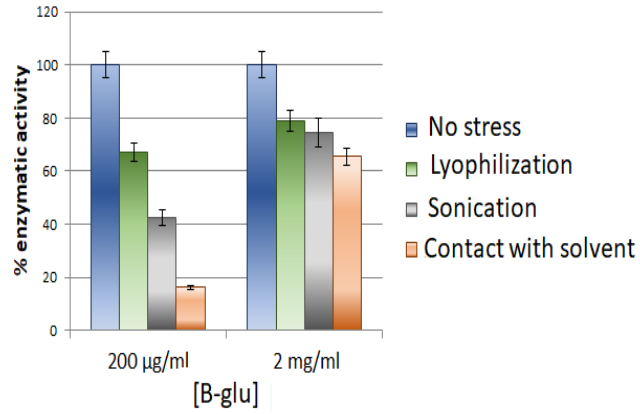
DRUG

Anti Prion Diseases
T3, Ibuprofen – inflamma
KVLFF peptide – Alzheir
Curcumin
Cholesterol - Huntingtor
DNA
Anti inflammatory drugs
Enzymes

Critical Points in **Enzyme Formulation with PLGA NPs**



THE EFFECT OF FORMULATIVE STRESS FACTOR ON ENZYME ACTIVITY



Enzyme-Loaded NPs 2.0 (Iduronate 2-sulfatase)

Samples	Z-Average [a] nm	PDI [a]	ζ-pot [a] mV	mg of IDS/100 mg NPs	EE% [b]
u-NPs-IDS	205 (12)	0.190 (0.02)	−36 (3)	3.1 (0.3)	31%
g7-NPs-IDS	203 (11)	0.214 (0.03)	−34 (5)	1.5 (0.9)	15%
g7-NPs	197 (12)	0.182 (0.01)	−32 (4)	/	/

International Journal of *Molecular Sciences*

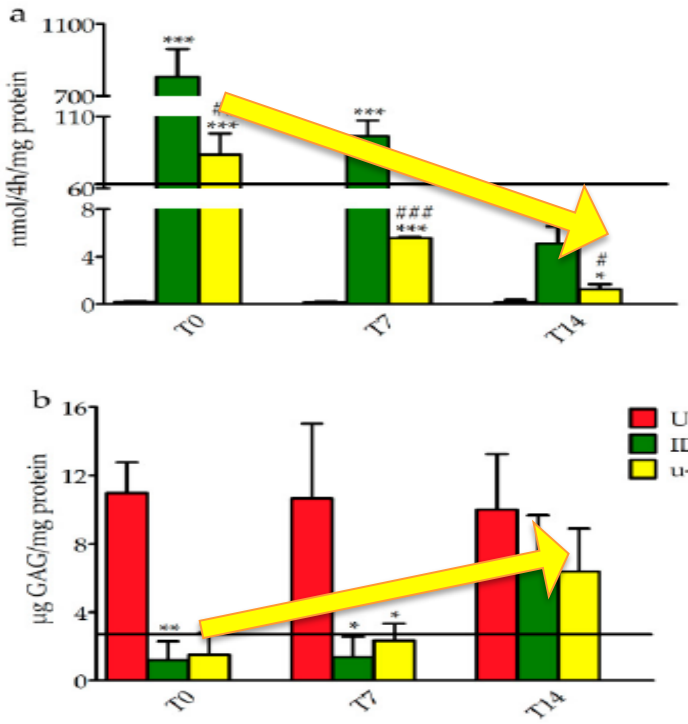
MDPI

Article

Targeting Brain Disease in MPSII: Preclinical Evaluation of IDS-Loaded PLGA Nanoparticles

Laura Rigon [1,2,†], Marika Salvalaio [2,3,†], Francesca Pederzoli [2,4], Elisa Legnini [1,2], Jason Thomas Duskey [4], Francesca D'Avanzo [1,2], Concetta De Filippis [1,2], Barbara Ruozi [4], Oriano Marin [5], Maria Angela Vandelli [4], Ilaria Ottonelli [4], Maurizio Scarpa [1,2], Giovanni Tosi [4] and Rosella Tomanin [1,2,*]

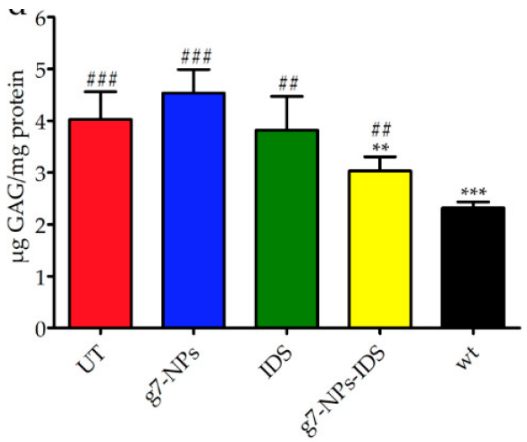
7 days *IN VITRO* on Fibroblasts from MPSII patients

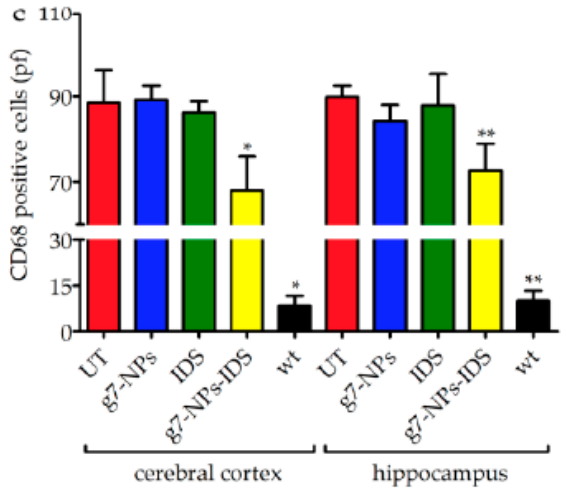


Induced **Enzyme activity**

Reduction of **GAG content**

6 weeks *IN VIVO* short-term study in MPSII mice





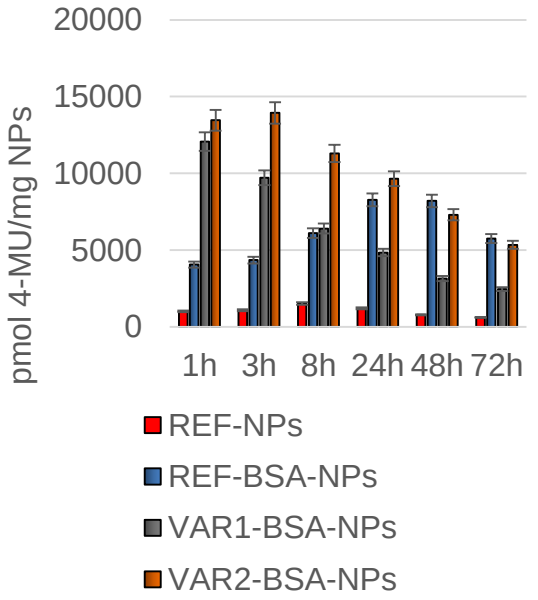
Significant reduction of GAG deposits in liver and brain tissues

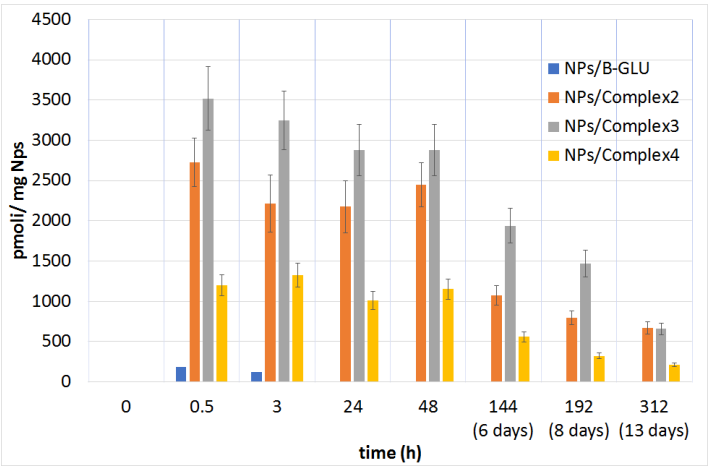
Reduction of some neurological pathological markers as neuroinflammation

Stabilizer Effects and Characterization

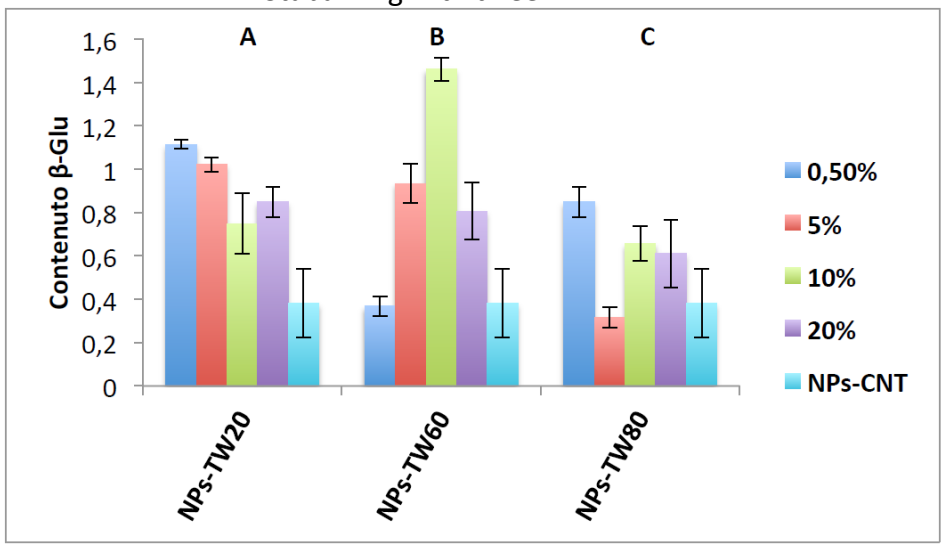
Chemical-physical properties of BSA:B-GLU NPs

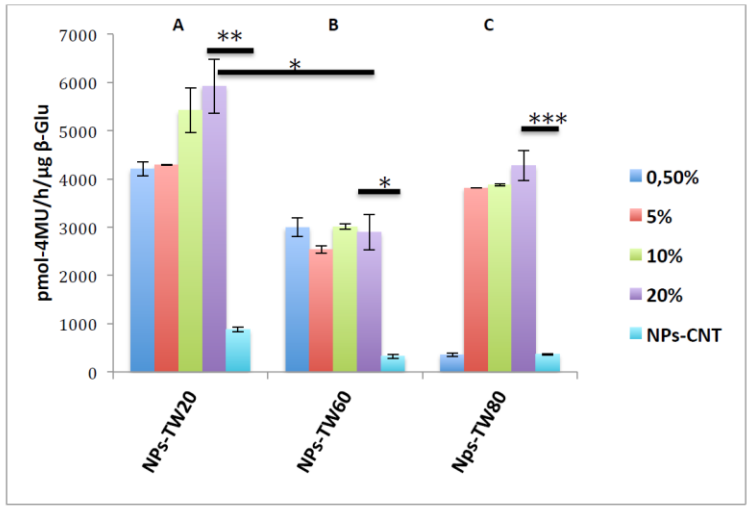
Samples (BSA:B-glu mol:mol)	Z-Average nm (S.D.)	PDI[a] (S.D.)	D(i)50[b] nm (S.D.)	D(i)90[b] nm (S.D.)	AFM diameter nm (S.D.)	ζ-pot m V (S.D.)	Yield%[c] (S.D.)	LC% (S.D.)	EE% (S.D.)
NPs	194 (17)	0.06 (0.02)	200 (16)	311 (20)	320 (47)	-21 (3)	86.2 (2.1)		
NPs B-GLU	199 (28)	0.17 (0.04)	221 (26)	376 (42)	311 (69)	-24 (6)	77.6 (3.3)	0.6 (0.1)	5.7 (0.9)
NPs/Complex1 (2:1)	234 (19)	0.21 (0.01)	227 (12)	365 (25)	302 (34)	-19 (2)	69.2 (4.1)	0.7 (0.3)	6.8 (2)
NPs/Complex2 (10:1)	222 (17)	0.11 (0.03)	208 (19)	332 (21)	365 (76)	-25 (3)	59.1 (2.6)	3.1 (1.9)	31 (7)
NPs/Complex3 (20:1)	243 (31)	0.14 (0.02)	215 (11)	339 (14)	375 (64)	-20 (3)	51.1 (2.2)	3.9 (1.4)	38.7 (4)
NPs/Complex4 (40:1)	266 (72)	0.31 (0.09)	253 (19)	443 (16)	/	-22 (7)	33.6 (7.2)	1.2 (0.4)	11 (5)





Stabalizing with tween

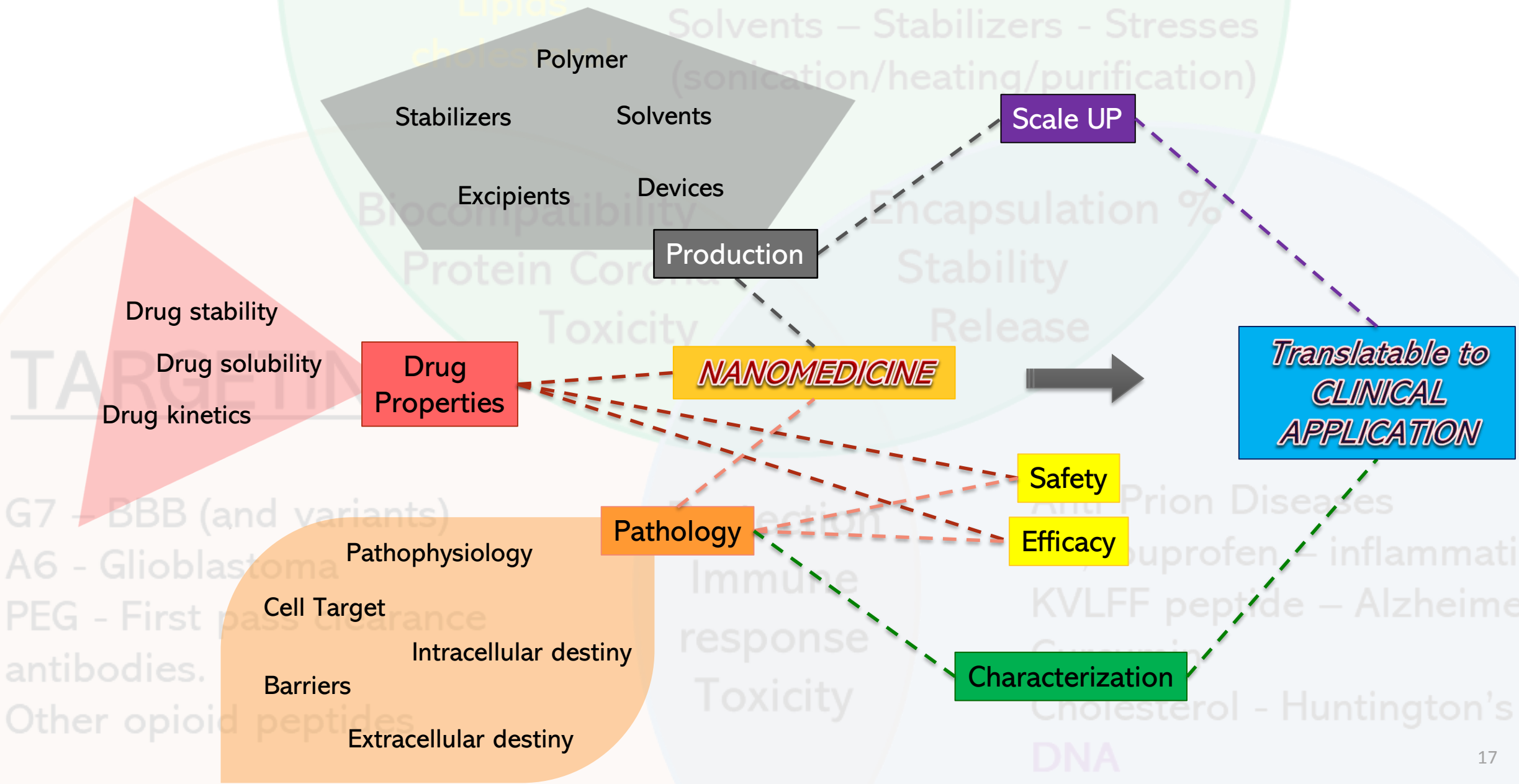




Two distinct modes of action
1) increase NP activity by increasing enzyme content
2) marginally increasing content but increasing enzyme activity through stabilization?

Work in cooperation*

Completed Smart NMED Vectors From design to Product



Application of Brain Targeted Nanomedicines in Brain Disease Models

Huntington's Disease (preclinical)
#UniMi

Neurometabolic Lysosomal Storage Diseases (preclinical)
 # Padova University

Alzheimer's Disease (preclinical)
#Ulm University

Brain Injury (preclinical)
#Uppsala University

Targeting Neuroinflammation

Prion Disease
 # Mario Negri Institute

Epilepsy
#UniFi & Meyer Hospital

Brain Cancer
#University of Princeton & University of Anger
#University of Limerick and the Bernal Institute

Narcolepsy
#University of Bologna

TELETHON RESEARCH PROJECTS
Impact on pathology
European Task Force on Brain and Neurodegenerative Lysosomal Storage Diseases

Rescue of diseased phenotype

nanomedicine
EUROPEAN TECHNOLOGY PLATFORM

ACKNOWLEDGEMENTS

- Prof.ssa Maria Angela Vandelli
- Prof. Flavio Forni
- Prof. Giovanni Tosi
- Prof.ssa Barbara Ruozi
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