

FIG. 1

- Bifunctional molecule

1. Cross blood brain barrier

2. Bind target protein

3. Remove target protein

peripheral sink
lysosomal degradation
- Modular system to develop platform for degradation of neurological proteins
- Low density lipoprotein receptor related protein 1 (LRP1)

LRP1 mediated transcytosis and degradation

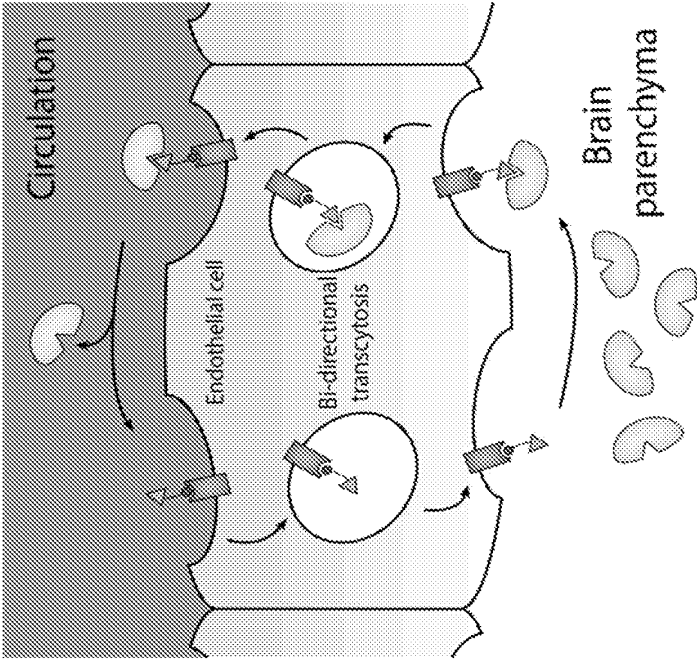


FIG. 2

Name	Sequence	Site for ligand attachment
Angiopep-2	TFYGGSRGKRNNFKTEEY-OH (or -NH ₂)	N term, K10, K15
L57	TWPKHFDKHTFYSSILKLGKH-OH	N term Cys added to N term Lys added to N term
Rap12	EAKIEKHNNHYQK-NH ₂	Side chain- Cys added to C term Lys added to C term
Rap22	EAKIEKHNNHYQKQLEIAHEKLR-NH ₂	Side chain- Cys added to C term Lys added to C term
ApoE (141-155)	LRKLRKRLLRDADDLLRKLRKRLLRDADDL-NH ₂	N term With linker
ApoE 130-149	TEELRVRLASHLRKLRKRLL-NH ₂	N term With linker

FIG. 3



To target extracellular tau

Name	Zipper Target	Peptide Sequence
VY-WIW	Class 3	SVWIWYE
IN-M4	Interface B	DVWIINKLK

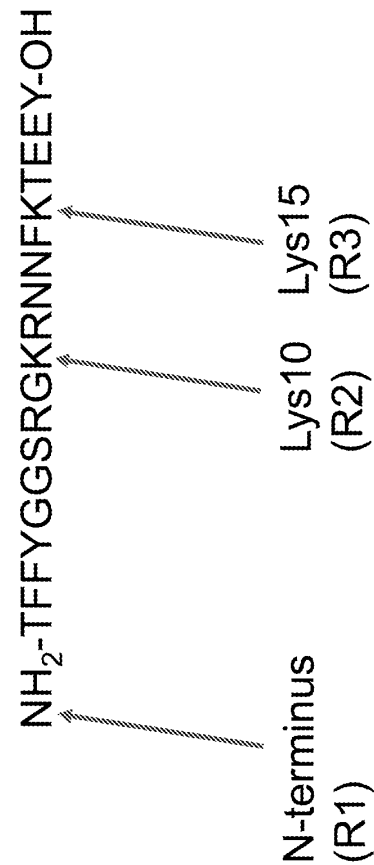
N- or C-term attachment

To target amyloid beta (bold)

Name	Sequence
NCAM1 (N)	MLRTKDLIWTLFFFLGTAVS-NH ₂
N-Pr	MLRTKDLIWTLFFFLGTAVS- KKRPPK P-NH ₂
N-Aβ	MLRTKDLIWTLFFFLGTAVS- KKLVFF -NH ₂

N- or C- term attachment

FIG. 4



Three sites tolerate modifications-

can be used for monitoring trafficking or protein targeting

Angiopep-2 nomenclature “R1.R2.R3 Angiopep-2”

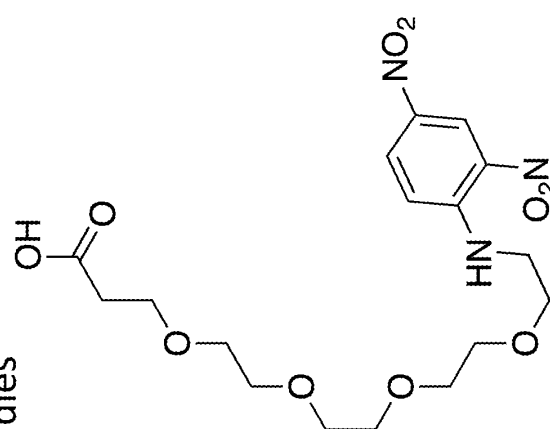
Ac- acetylated

Rho- rhodamine fluorescent label

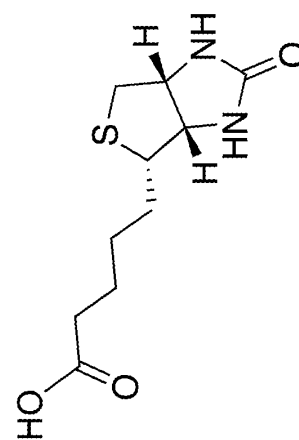
DNP- dinitrophenyl

FIG. 5

Proof-of-concept Studies



DNP



Biotin

FIG. 6

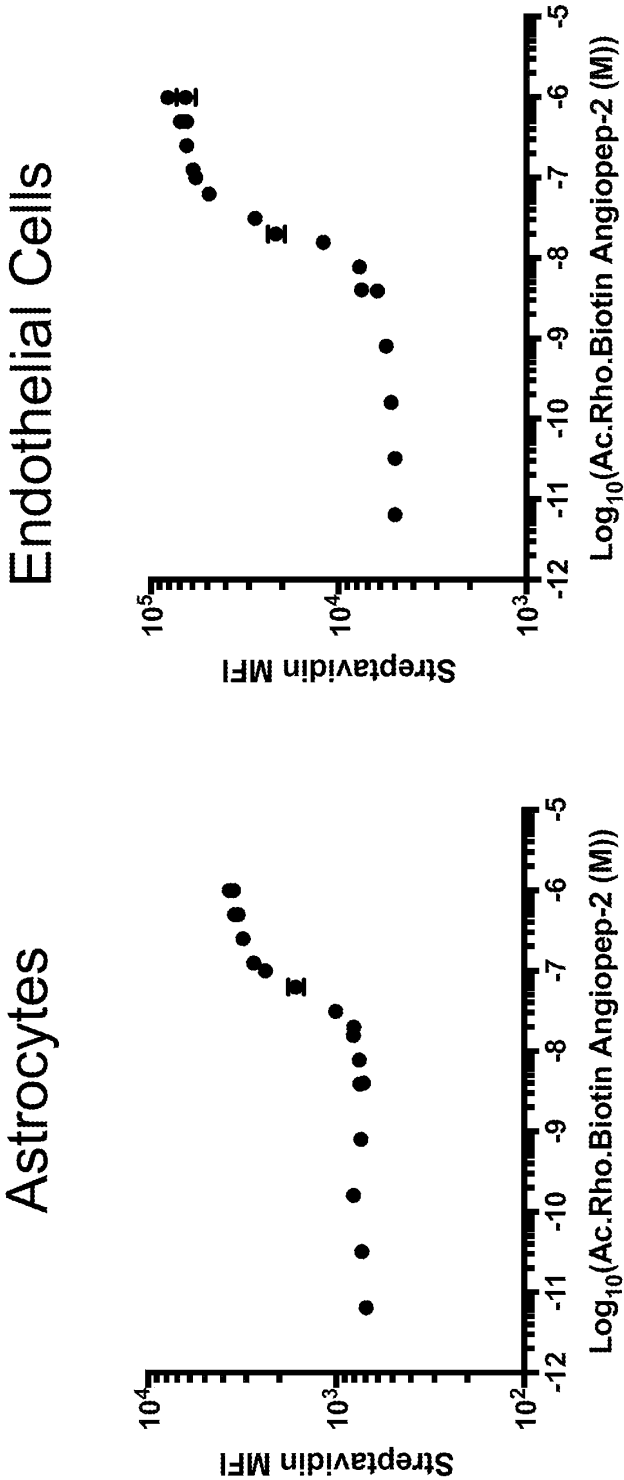


FIG. 7

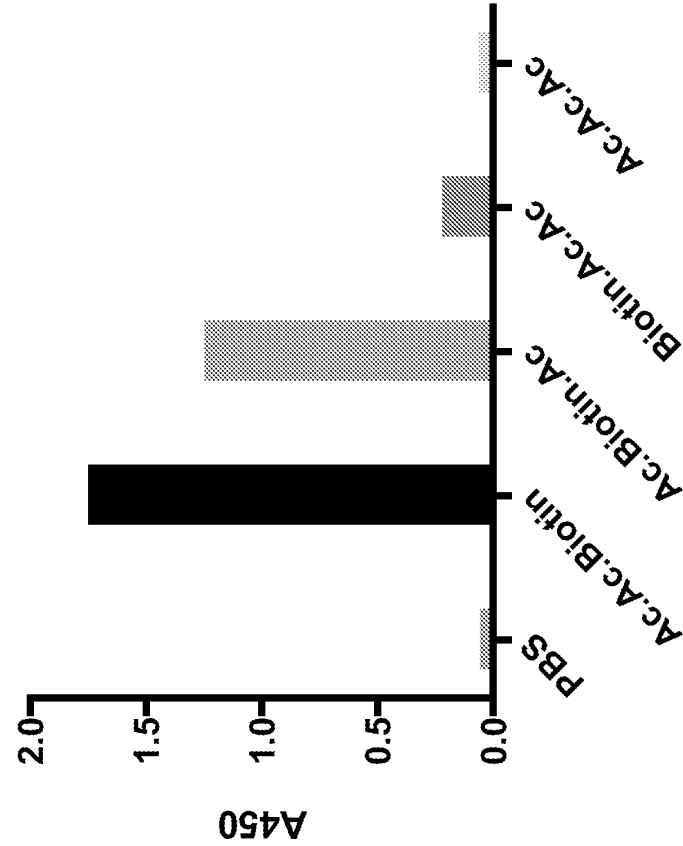


FIG. 8

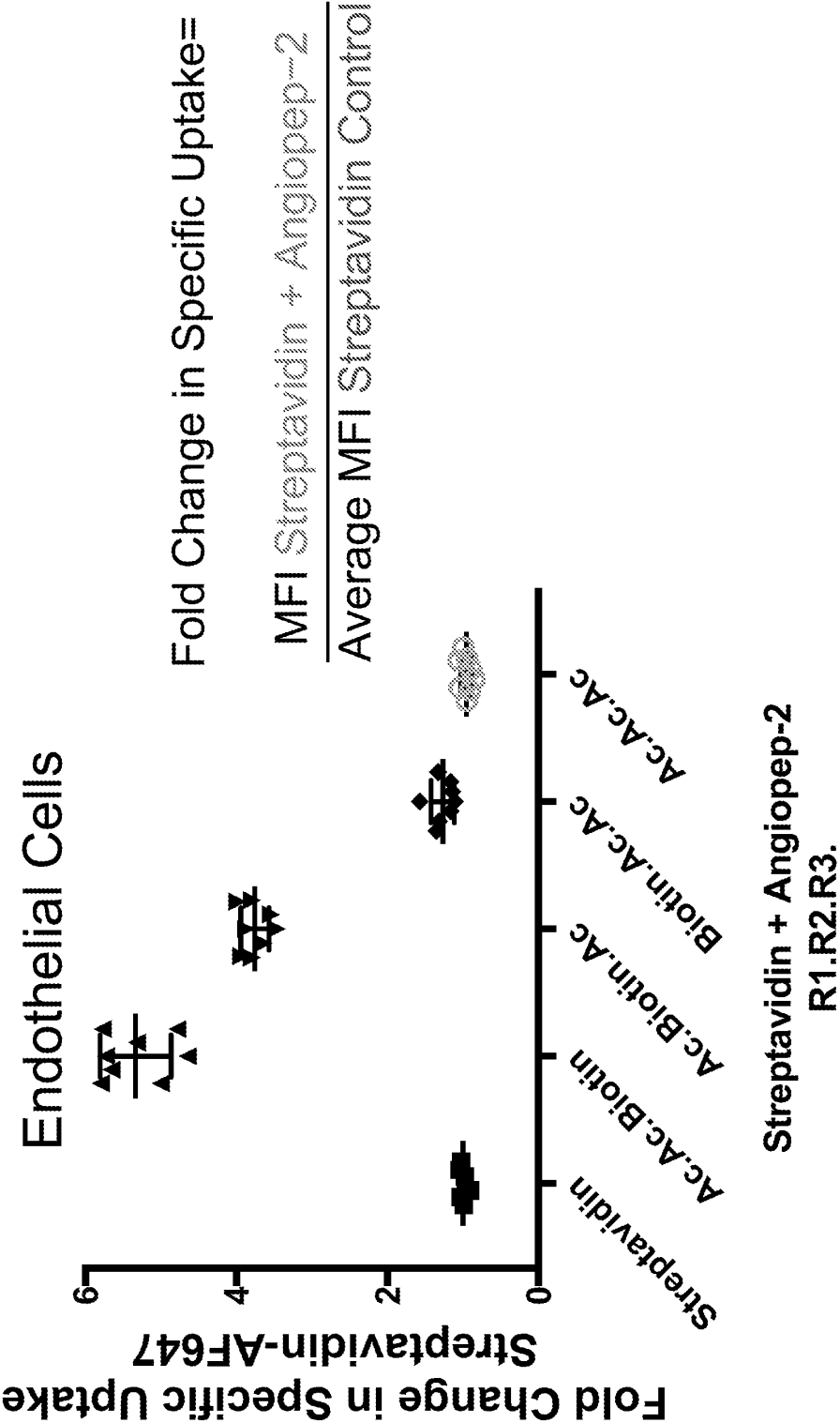


FIG. 9

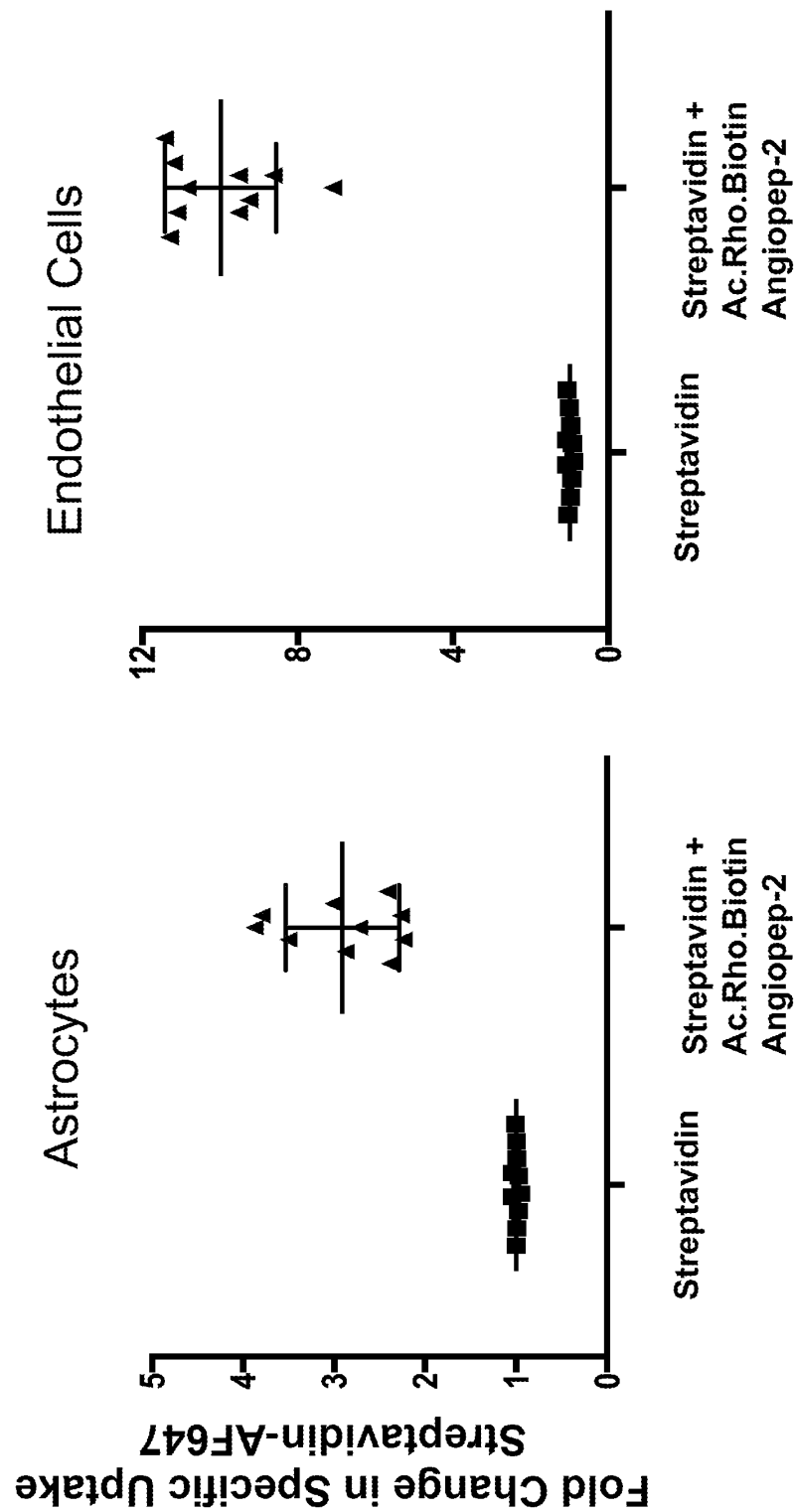


FIG. 10

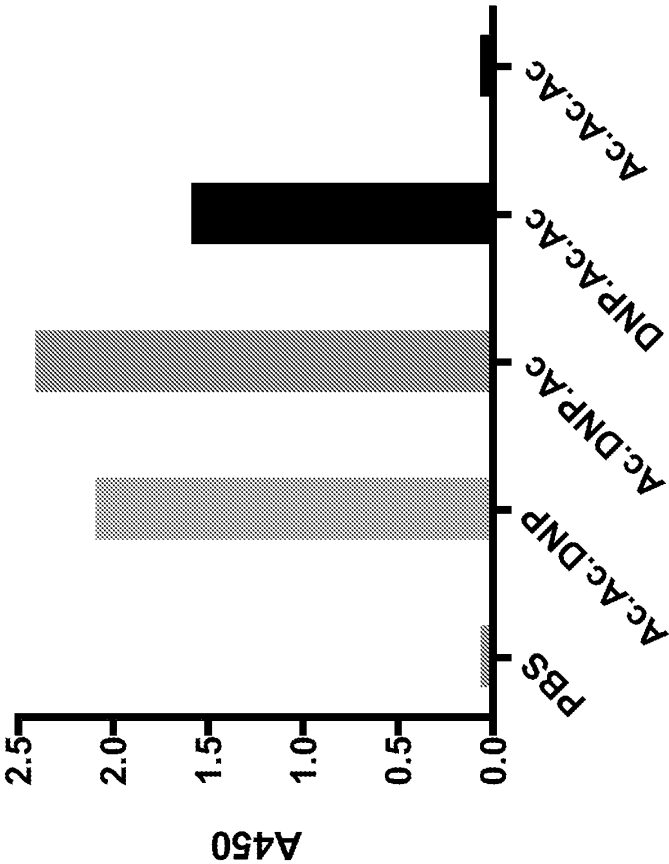


FIG. 11

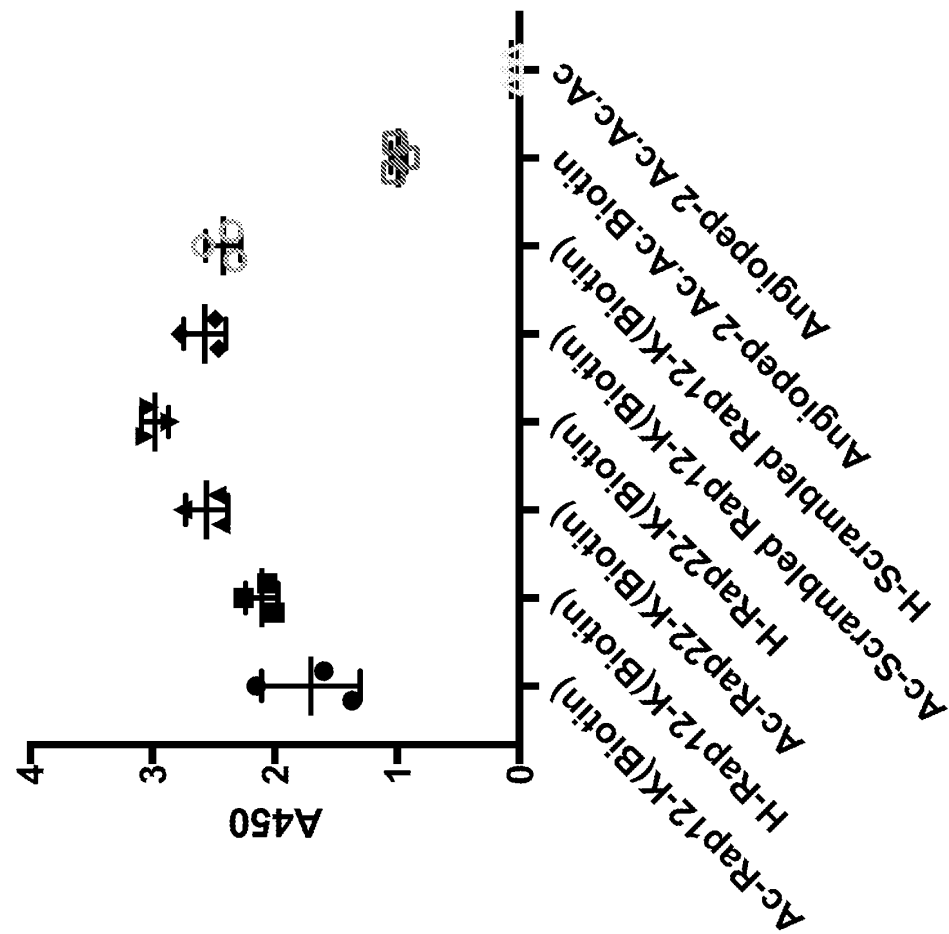


FIG. 12

Ac.Ac.Biotin Angiopep-2 mediated Degradation of Streptavidin AF488
Time Course and Titration in Endothelial Cells

Lane	1	2	3	4	5	6	7	8
Streptavidin AF488	+	+	+	+	+	-		0.5 ng Purified strept- avidin
Angiopep-2	+	+	+	+	+	-		
[Ang] (nM)	0	8	40	200	1000	0		

Lane	1	2	3	4	5	6	7	8
Streptavidin AF488	+	+	+	+	+	-		0.5 ng Purified strept- avidin
Angiopep-2	+	+	+	+	+	-		
[Ang] (nM)	0	8	40	200	1000	0		

