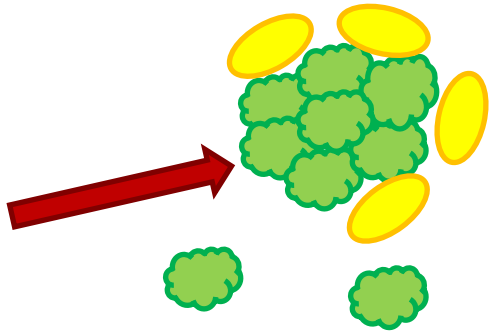
Herpes simplex virus gD glycoprotein derived peptides as potential drug carriers

Katalin Uray PhD

MTA-ELTE Research Group of Peptide Chemistry

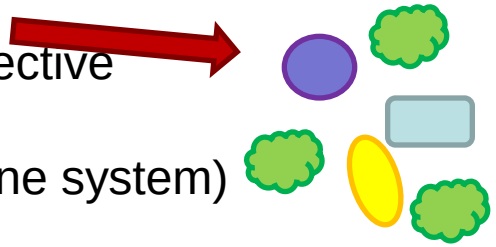
22 March 2018

Targeted therapy



Cancer therapies:

Surgery
Radiotherapy } local treatment

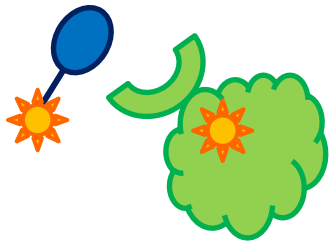


Chemotherapy — generally not very selective

Immune therapy (antibodies, boosting own immune system)

Targeted therapy

Drug molecules are specifically targeted to cells with certain features –
a special type of cell, a cell with certain receptors on its surface



Inspiration – viruses

Viruses

Virus: latin, poisonous

Meaning „Agent that causes infectious disease": first recorded in 1728

Louis Pasteur: could not find the causative agent of rabies – is it too small?

Charles Chamberland, 1884: filter with pores smaller than bacteria

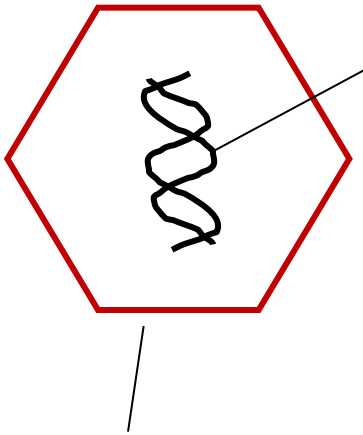
Dimitri Ivanovsky, 1892, tobacco mosaic virus: goes through filter: infectious after filtration – bacterial toxin?

Martinus Beijerinck, 1898, new form of infectious agent in the filtrate „contagium vivum fluidum” (soluble living germ) – re-intruducing the word „virus”

Growing viruses first on plant and animal hosts, then on tissue cultures (1906)

Knoll and Ruska, 1931: first electron microscopy image of viruses

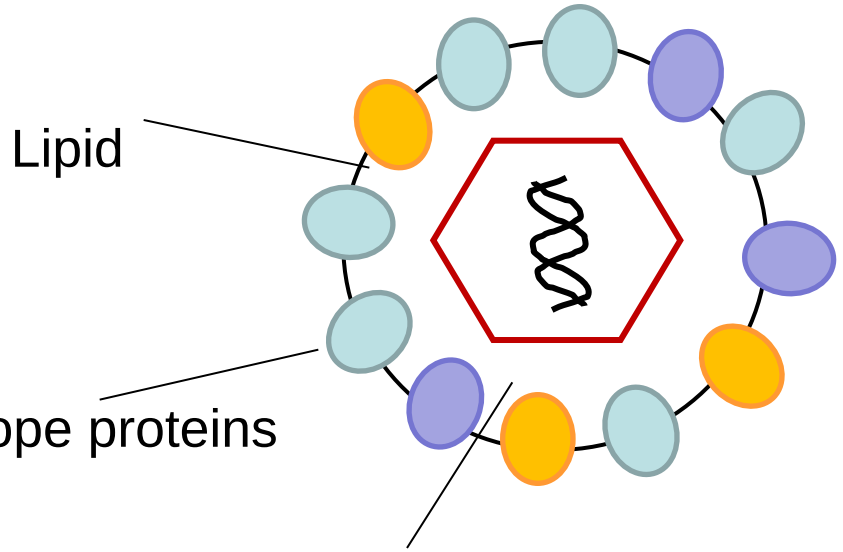
Viruses



DNA or RNA
Single stranded or double stranded
or ds and ss regions
Linear, circular or segmented
Positive sense, negative sense, ambisense

Protein capsid

Enveloped virus



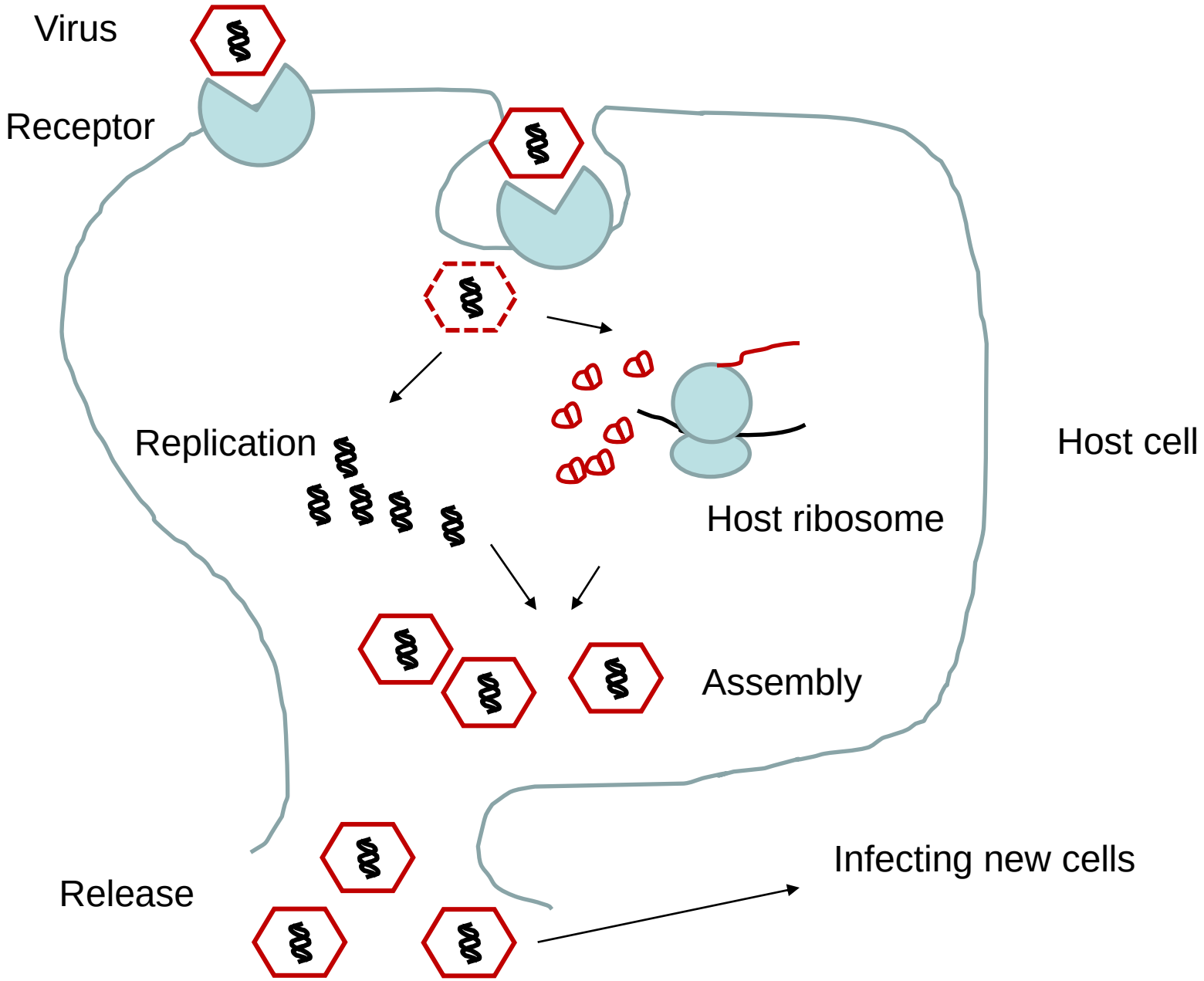
Lipid

Envelope proteins

Tegument

Size: cca 20 – 500 nm
Genome size: cca 2 000 – 2 000 000 bp
Shape: icosahedral, rod-like, spherical

Virus „life” cycle



Baltimore classification (David Baltimore, Nobel prize)

I: dsDNA viruses (e.g. Adenoviruses, Herpesviruses, Poxviruses)
II: ssDNA viruses (+ strand or "sense") DNA (e.g. Parvoviruses)
III: dsRNA viruses (e.g. Reoviruses)
IV: (+)ssRNA viruses (+ strand or sense) RNA (e.g. Picornaviruses, Togaviruses)
V: (−)ssRNA viruses (− strand or antisense) RNA (e.g. Orthomyxoviruses, Rhabdoviruses)
VI: ssRNA-RT viruses (+ strand or sense) RNA with DNA intermediate in life-cycle (e.g. Retroviruses)
VII: dsDNA-RT viruses DNA with RNA intermediate in life-cycle (e.g. Hepadnaviruses)

International Committee on Taxonomy of Viruses (ICTV) classification

Order (-virales)
Family (-viridae)
Subfamily (-virinae)
Genus (-virus)
Species (-virus)

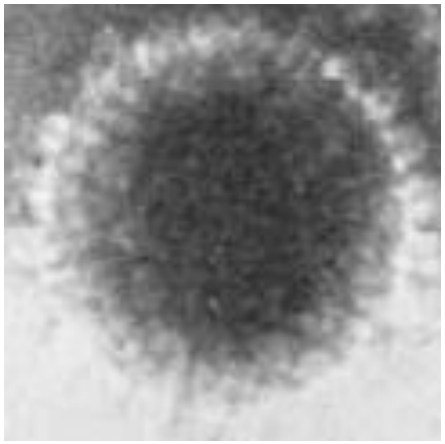
Herpesvirales, Herpesviridae

More than 130 species, 9 infecting humans (HHV)

α – herpesvirinae	β – herpesvirinae	γ – herpesvirinae
HSV-1, HSV-2, VZV HHV-1, HHV-2, HHV-3	HCMV, HHV-6A, HHV-6B, HHV-7 HHV-5,	EBV, KSHV HHV-4, HHV-8

HSV: *Herpes simplex* virus
VZV: *Varicella zoster* virus, chickenpox
HCMV: Human cytomegalovirus
EBV: Epstein-Barr virus
KSHV: Kaposi's Sarcoma associated Herpes virus

Herpes simplex virus 1 (HSV-1, HHV-1)



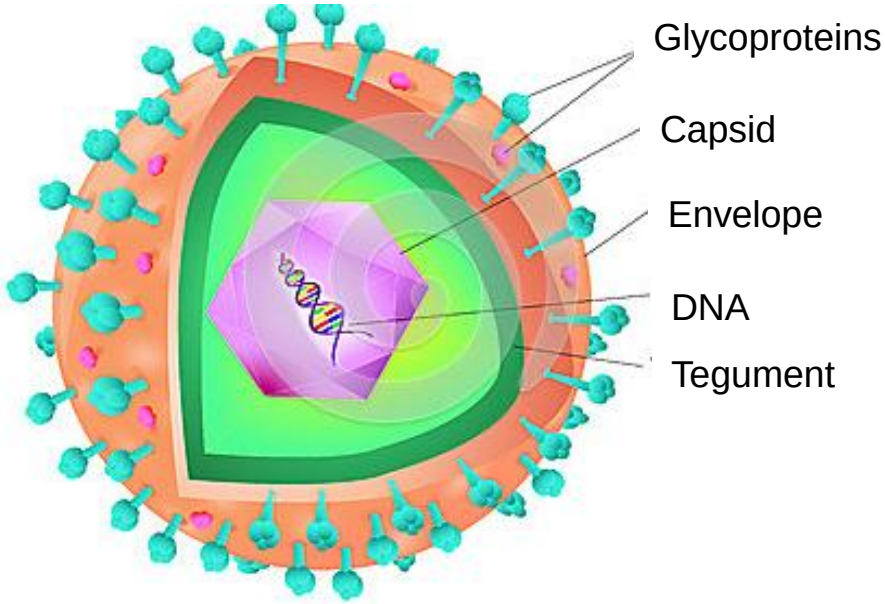
Herpesviridae, α-Herpesvirinae

Labial herpes (cold sores)

Complications:
meningitis, encephalitis
blindness

Frequent recurrence,
latency, in neurons
Evading the immune system

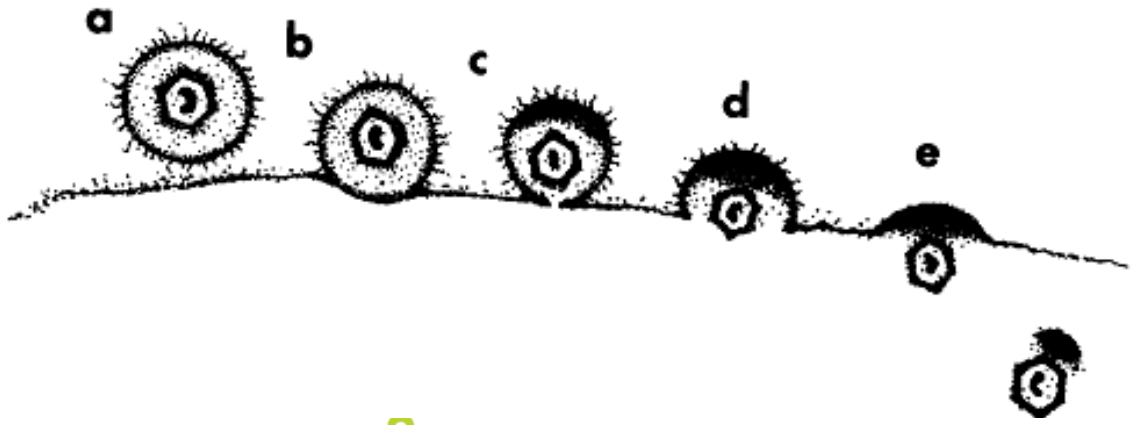
Treatment
Nucleoside derivatives
DNA inhibitors

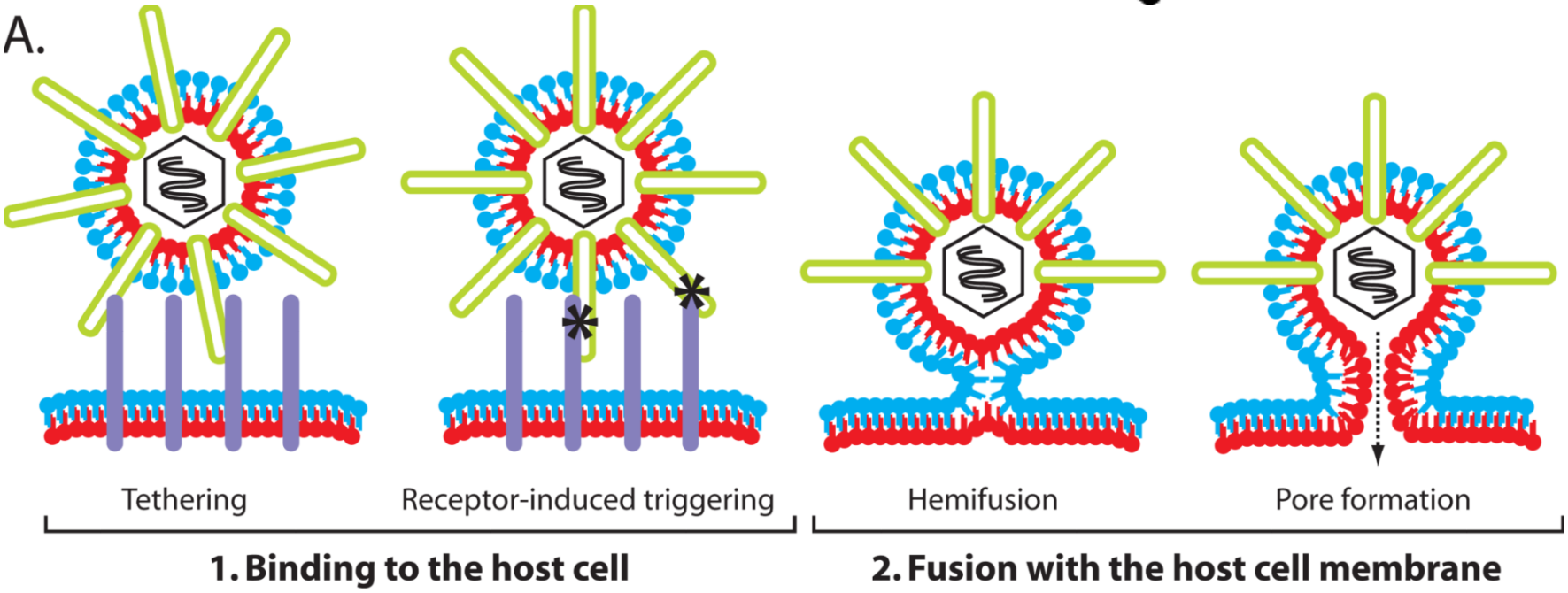


125 nm, 153 000 bp, ds DNA,
icosahedral capsid

http://www.dentalnotebook.com/human-herpesvirus-1/
http://bgr.com/2015/10/29/cancer-treatment-herpes-virus-t-vec/

Cellular entry of *Herpes simplex* virus

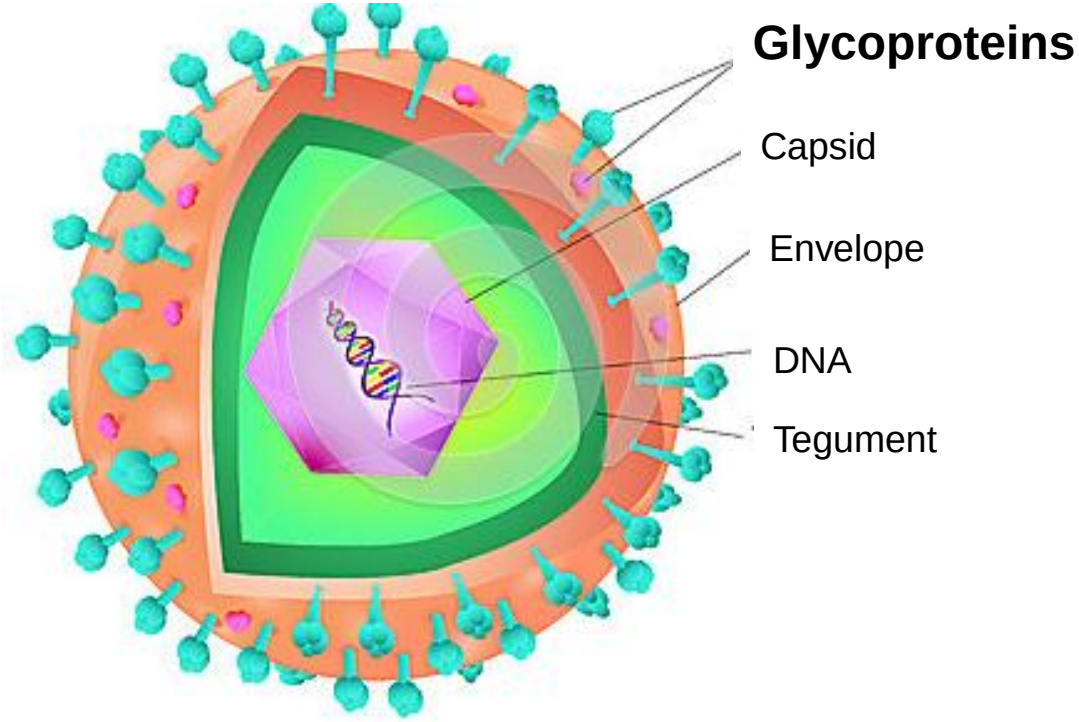




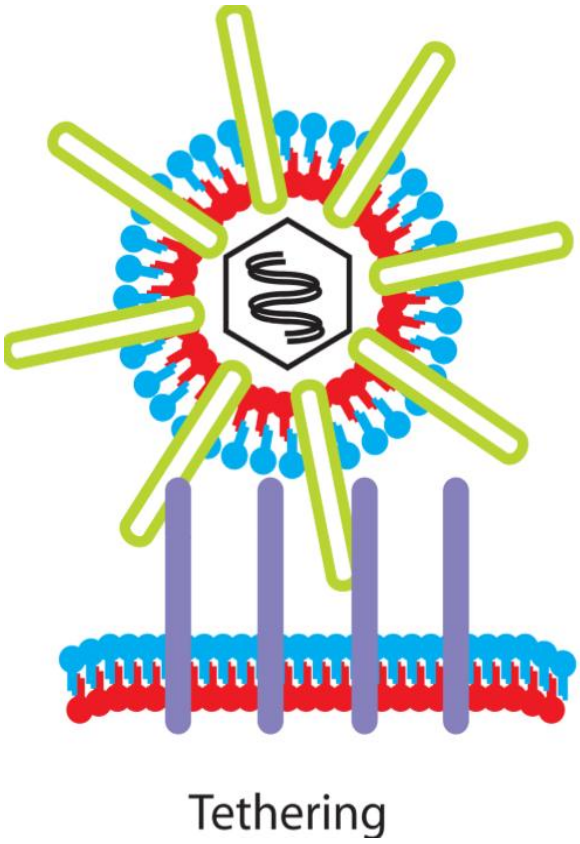
Fuller és Lee: J Virol 66(8): 5002-5012. (1992)

Connolly et al: Nat Rev Microbiol 9(5):369-381. (2011)

Participating glycoproteins in HSV entry



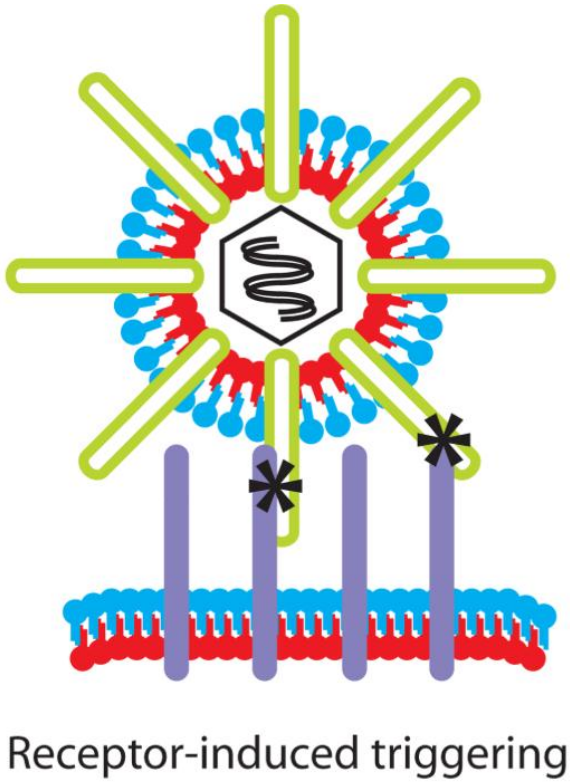
gB
gC
gD
gH/gL



Tethering

Concentrating viruses on the cell surface
Does not specifically trigger fusion

gB and gC interact with heparan sulfate



Receptor-induced triggering

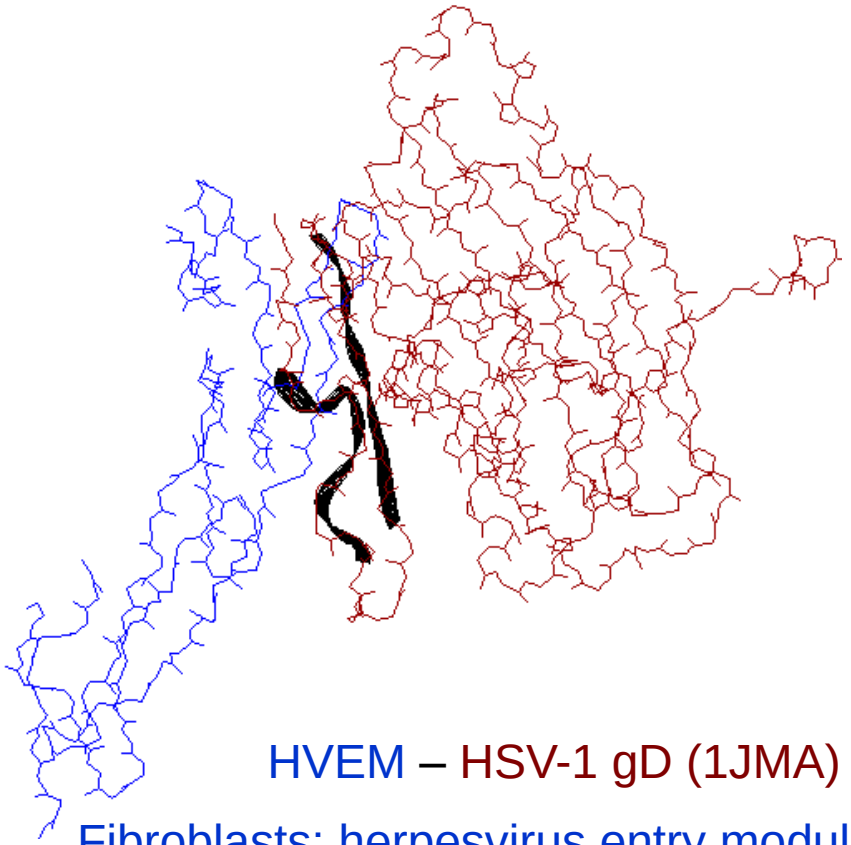
Receptor induced triggering

gD is the main receptor binding glycoprotein
Unique for *Herpes simplex* 1 and 2 viruses

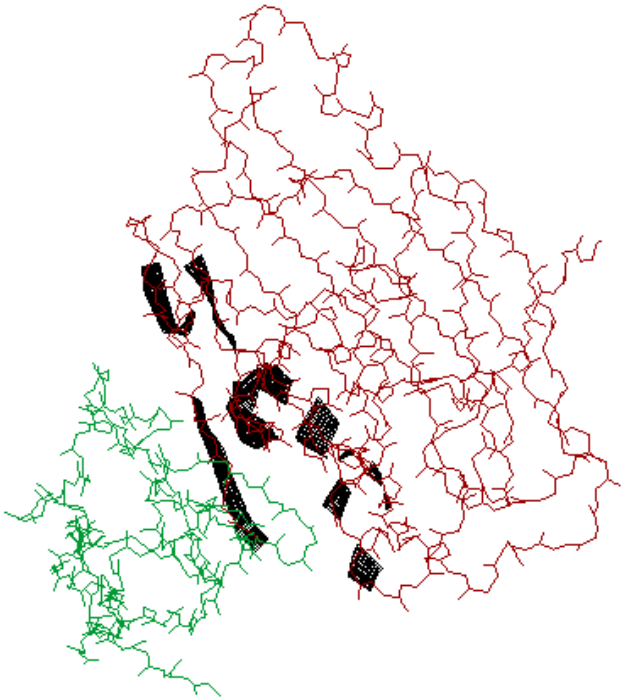
Receptors: HVEM (herpes virus entry modulator, immunomodulator, tumor necrosis factor receptor superfamily)

Nectin-1/2 (cell adhesion molecules)

Binding of HSV gD to HVEM and nectin receptors



HVEM – HSV-1 gD (1JMA)

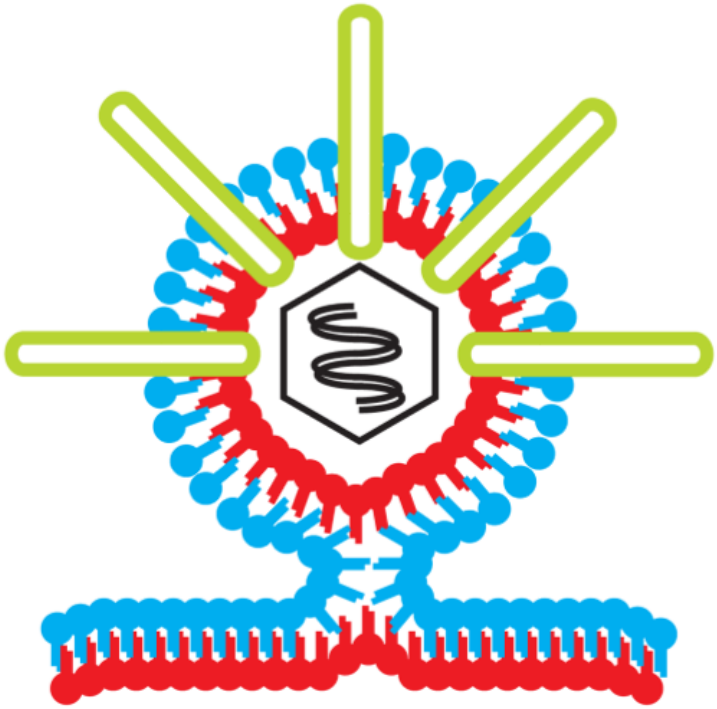


Nectin – HSV-1 gD (3U82)

Fibroblasts: herpesvirus entry modulator A (HVEM), immunomodulator, tumor necrosis factor receptor superfamily.

Neurons, keratinocytes, epithelial cells: nectin-1 adhesion protein

Carfí et al: Acta Crystallogr D Biol Crystallogr 58(Pt 5):836-838 (2002)
Di Giovine et al: PLoS Pathog 7(9):e1002277 (2011)

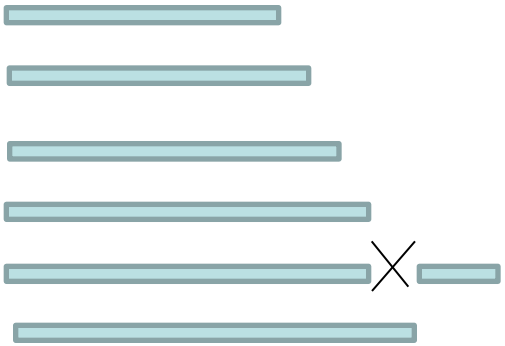


Hemifusion

Fusogenic glycoproteins
gB, gH/gL
(in other herpes viruses as well,
e.g. Epstein-Barr)

gH has amphiphil helices,
effecting pore formation

Structural requirements for gD for infection

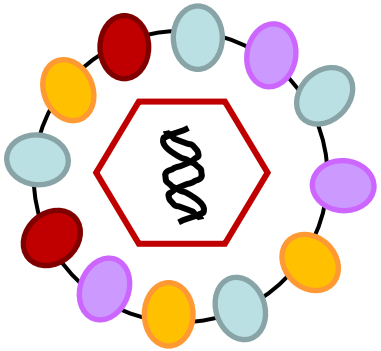


Mutant gD-s:	affinity to receptors
gD-243t	+
gD-250t	++
gD-275t	+++
gD-285t	+++
gD-Δ290-299t	+++
gD-306t	+

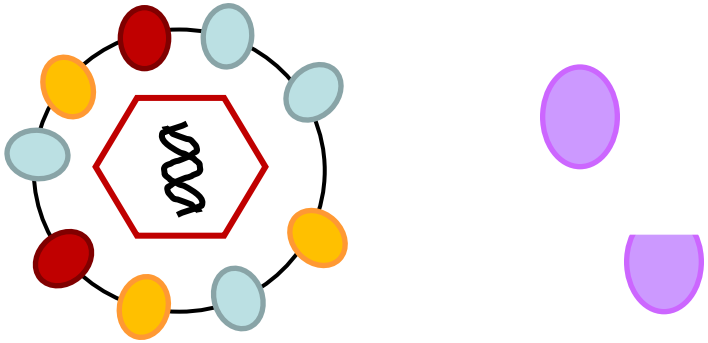
Milne et al, 2003, J Virol 77, 8962-8972
Krummenacher et al, 1998, J. Virol 72, 70-64-7074

Structural requirements for gD for infection

Can soluble gD substitute the virion-bound gD?



HSV wt



HSV gD null

gB
gC
gD
gH/gL

	BHK cells	Nectin 1-J cells
gD-243t	-	-
gD-250t	-	-
gD-260t	-	-
gD-275t	-	+
gD-285t	-	+++
gD-Δ290-299t	+++	+++
gD-306t	++	+++
	(hamster kidney, fibroblast,	nectin expressing cells)

Cocchi et al, 2004, PNAS 101(19):7445-7450

Structural requirements for gD for infection

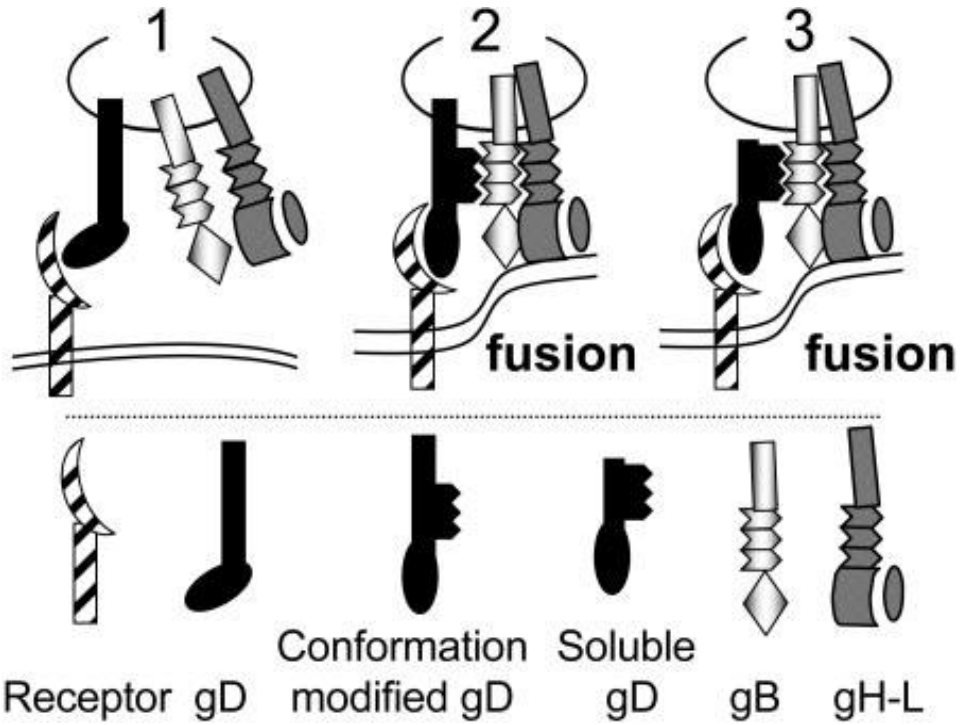
Receptor binding domain

Pro-fusion domain
260-307

TM, C-term:
Not needed for infection

Role of gD:
Not only receptor binding!

to form a tripartite complex
Switch of fusion glycoproteins to fusion-active state

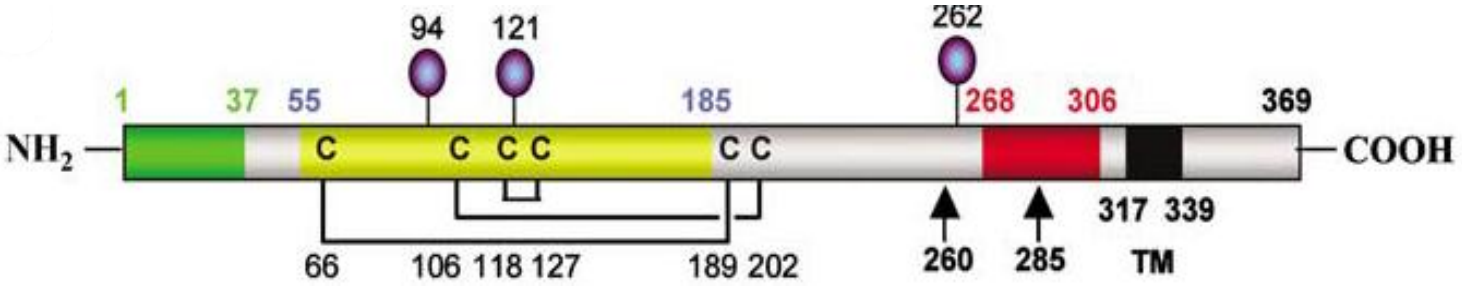


Cocchi et al, 2004, PNAS 101(19):7445-7450

2005: three X-ray structures,

gD 1-285
gD 1-316
gD 1-316 bound to HVEM

260-285 disordered, not visible in X-ray
C-terminal not resolved

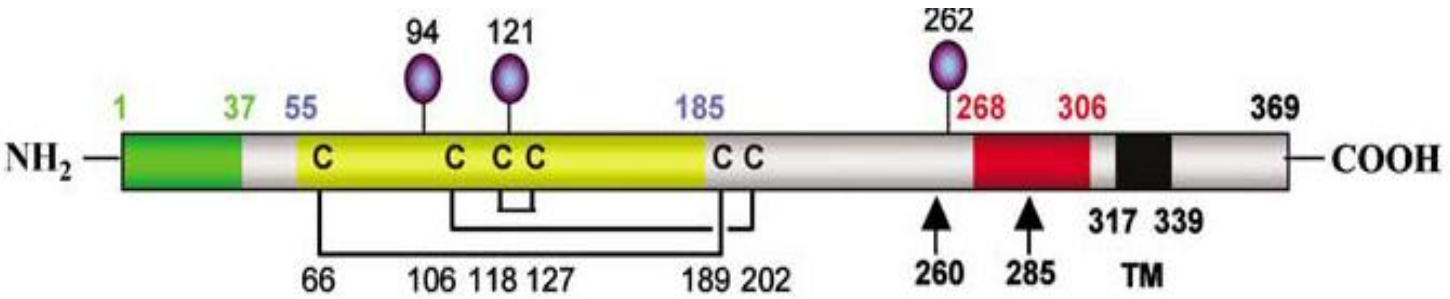


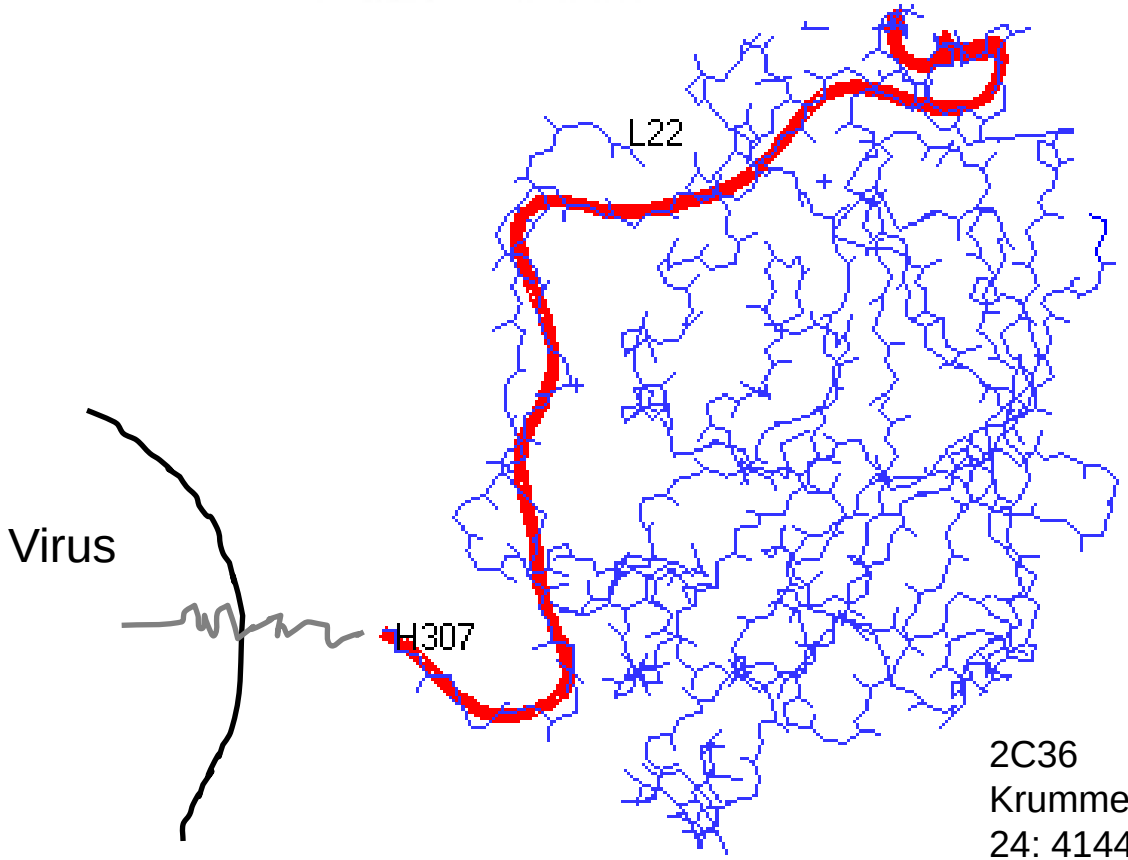
Receptor binding domain Pro-fusion domain TM, C-term
260-307

New: gD 1-306, + mutant (N-terminal not resolved)
pre-receptor binding conformer

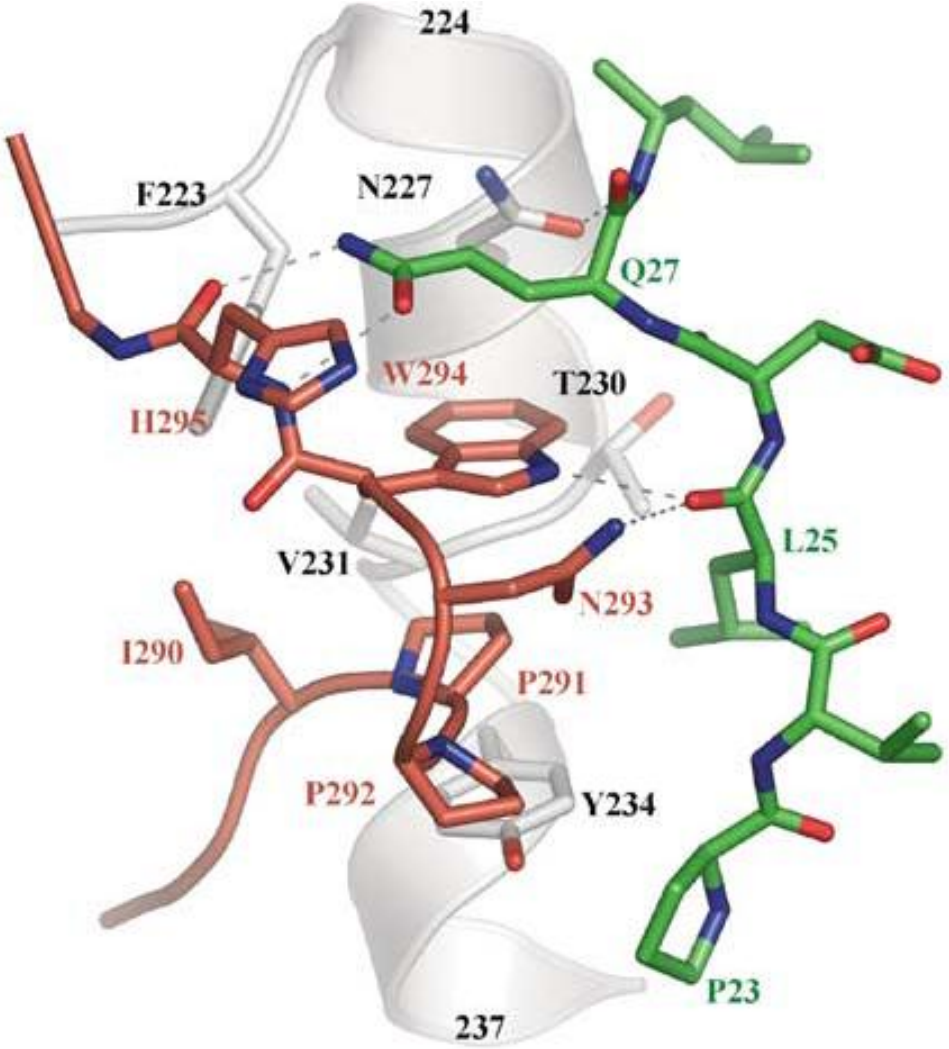
2C36 Krummenacher et al: EMBO J, 24: 4144-4153 (2005)

The structure of HSV-1 gD glycoprotein



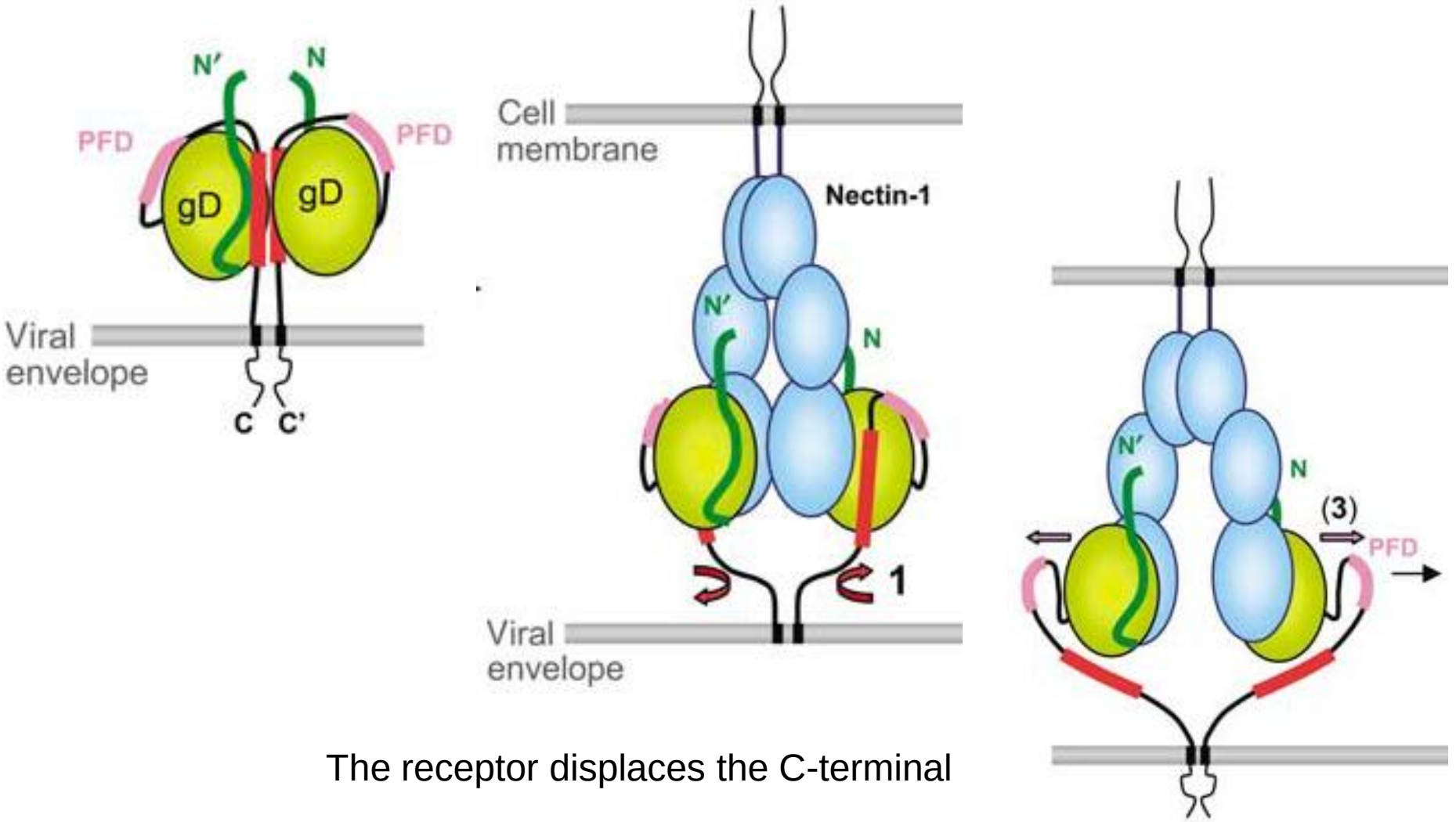


2C36
Krummenacher et al: EMBO J, 24: 4144-4153 (2005)



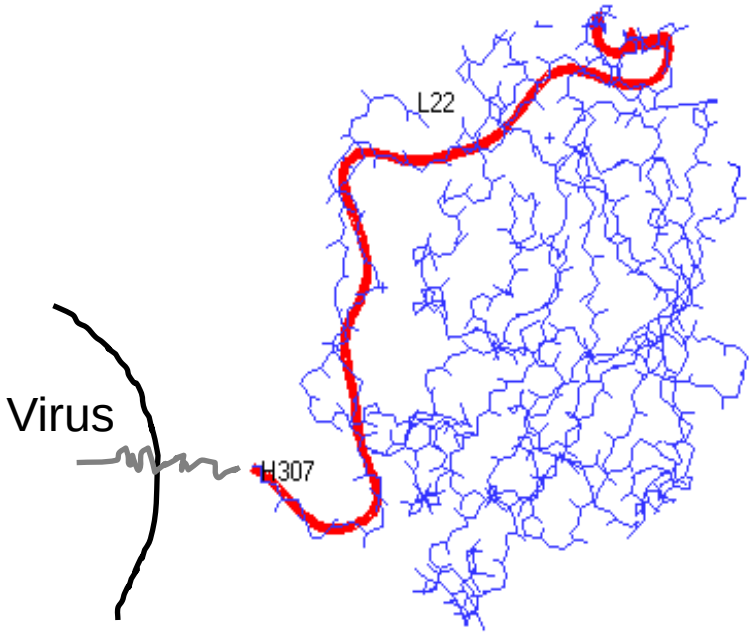
Flexible C-terminal,
Conformational equilibrium

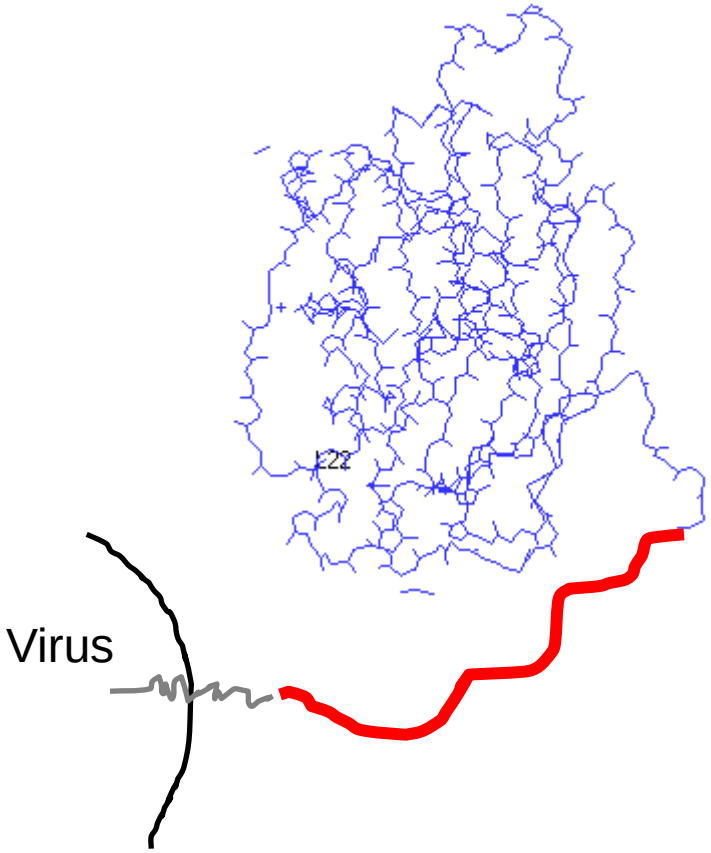
2C36 Krummenacher et al: EMBO J, 24: 4144-4153 (2005)



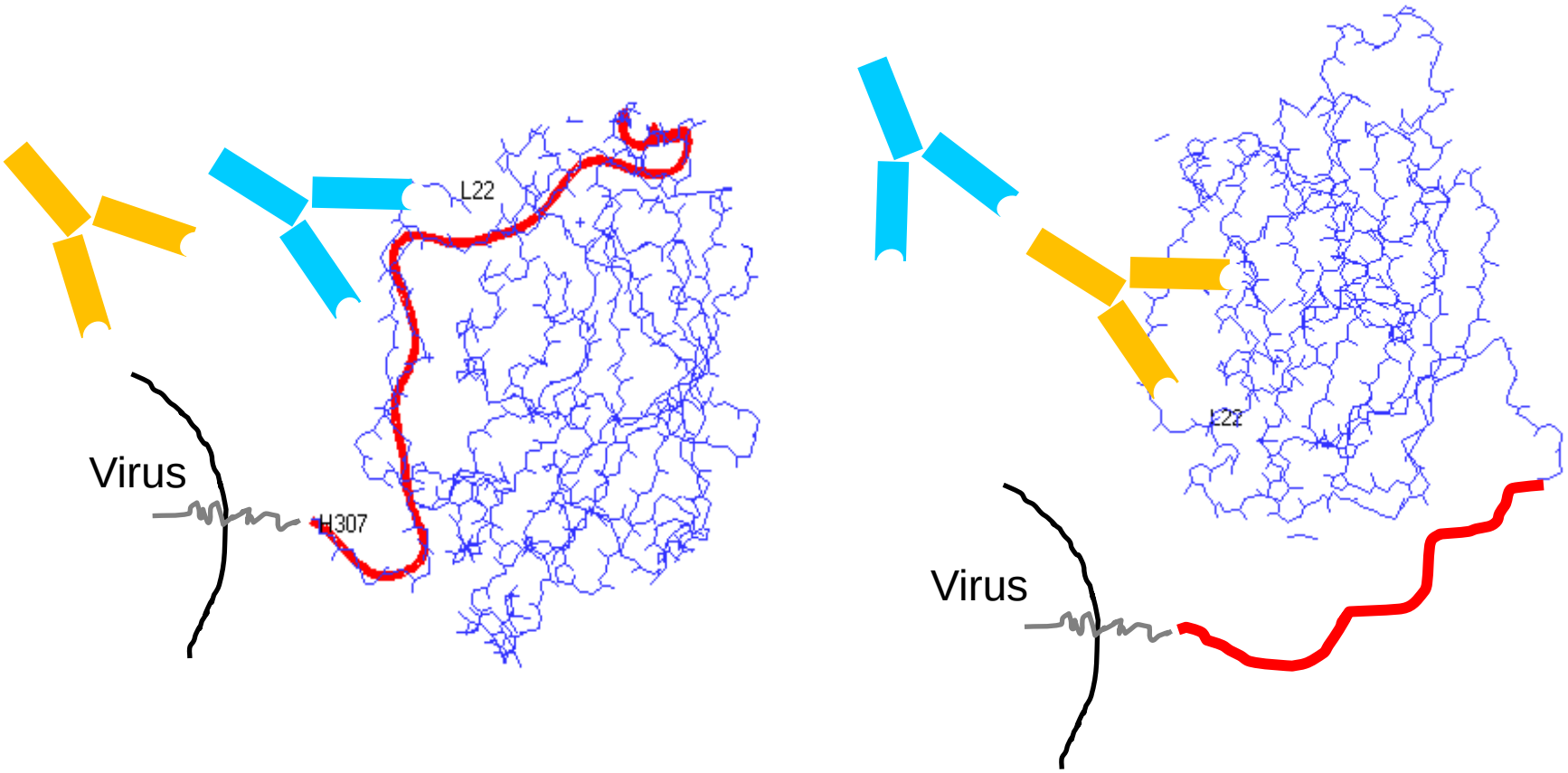
The receptor displaces the C-terminal

2C36 Krummenacher et al: EMBO J, 24: 4144-4153 (2005)



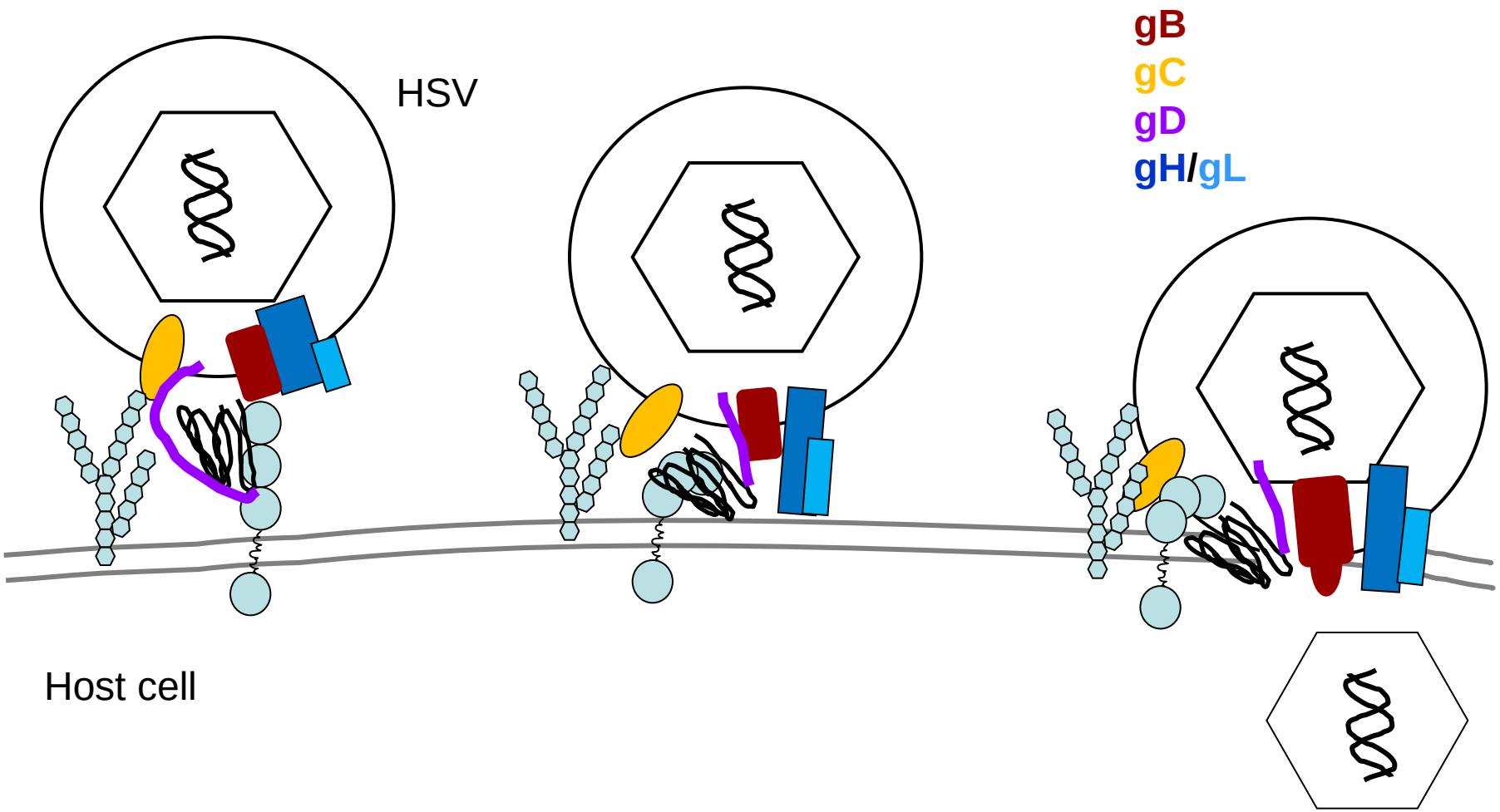


Masking both nectin and HVEM binding sites
Possibly gB and gH/gL binding sites are blocked



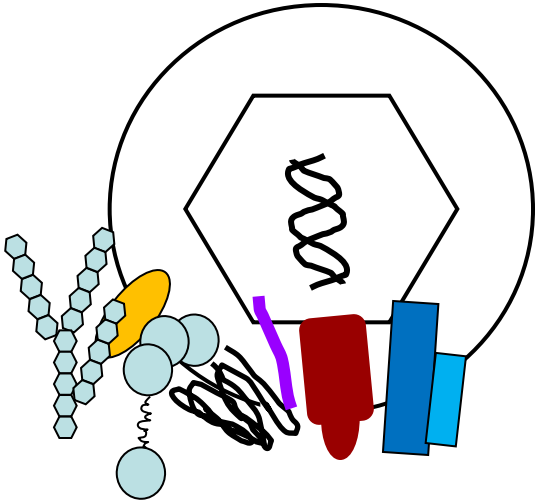
Protection against the host's immune system

Cellular entry of *Herpes simplex* virus



Connolly et al: Nat Rev Microbiol 9(5):369-381. (2011)

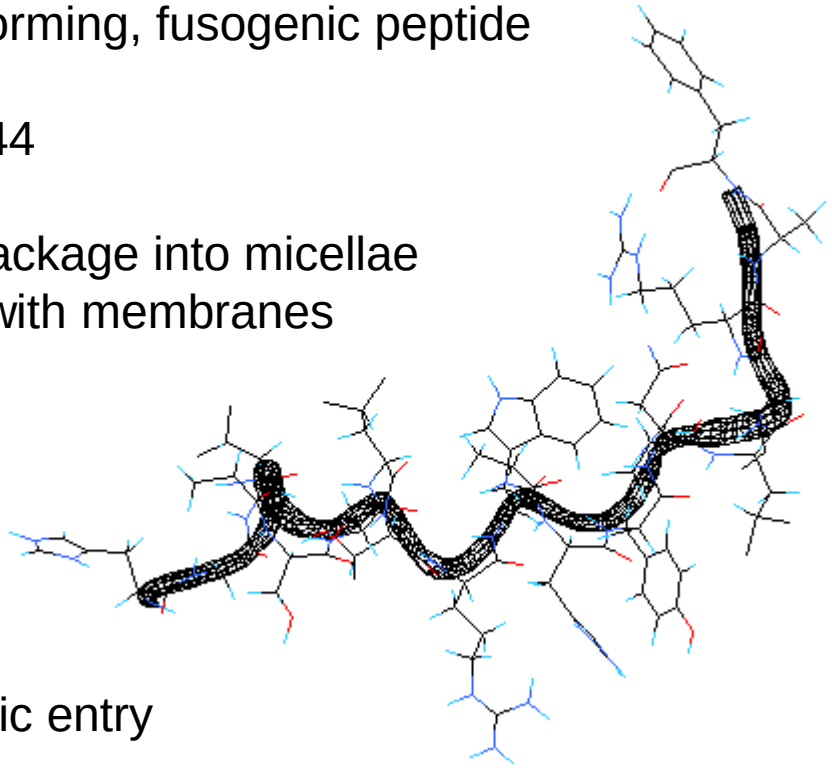
Using virus peptides for cellular targeting



gH pore forming, fusogenic peptide

gH 625-644

Easy to package into micellae
Interacts with membranes



Not specific entry

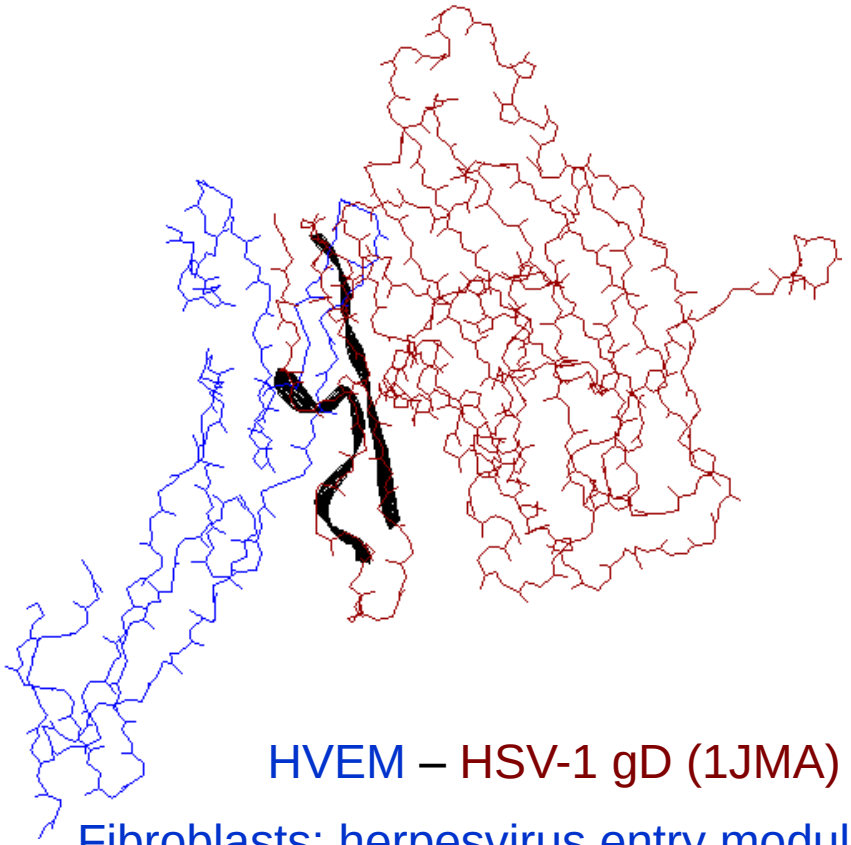
Galdiero et al, Biochemistry. 2012;51(14):3121-3128.

Our aims:

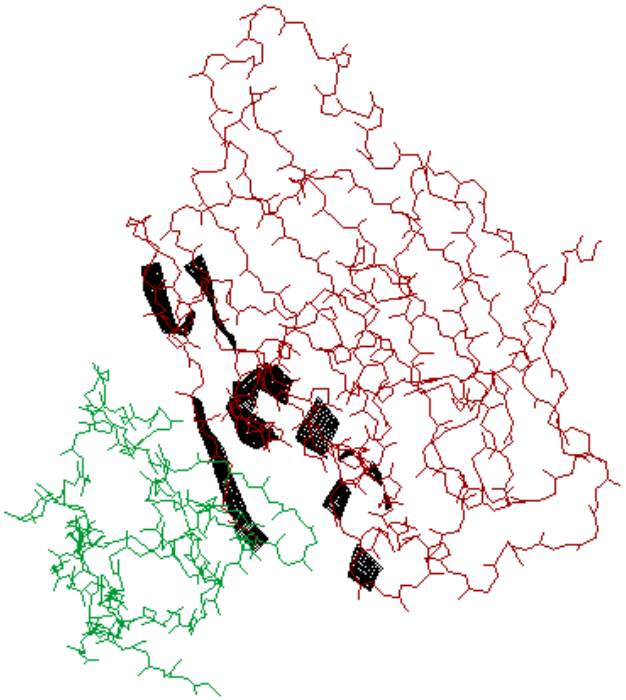
Finding synthetic peptides effectively and specifically internalising into cells carrying nectin or HVEM receptor,
with receptor mediated cellular uptake

Peptide sequences based on the receptor binding of HSV-1 gD

Binding of HSV gD to HVEM and nectin receptors



HVEM – HSV-1 gD (1JMA)



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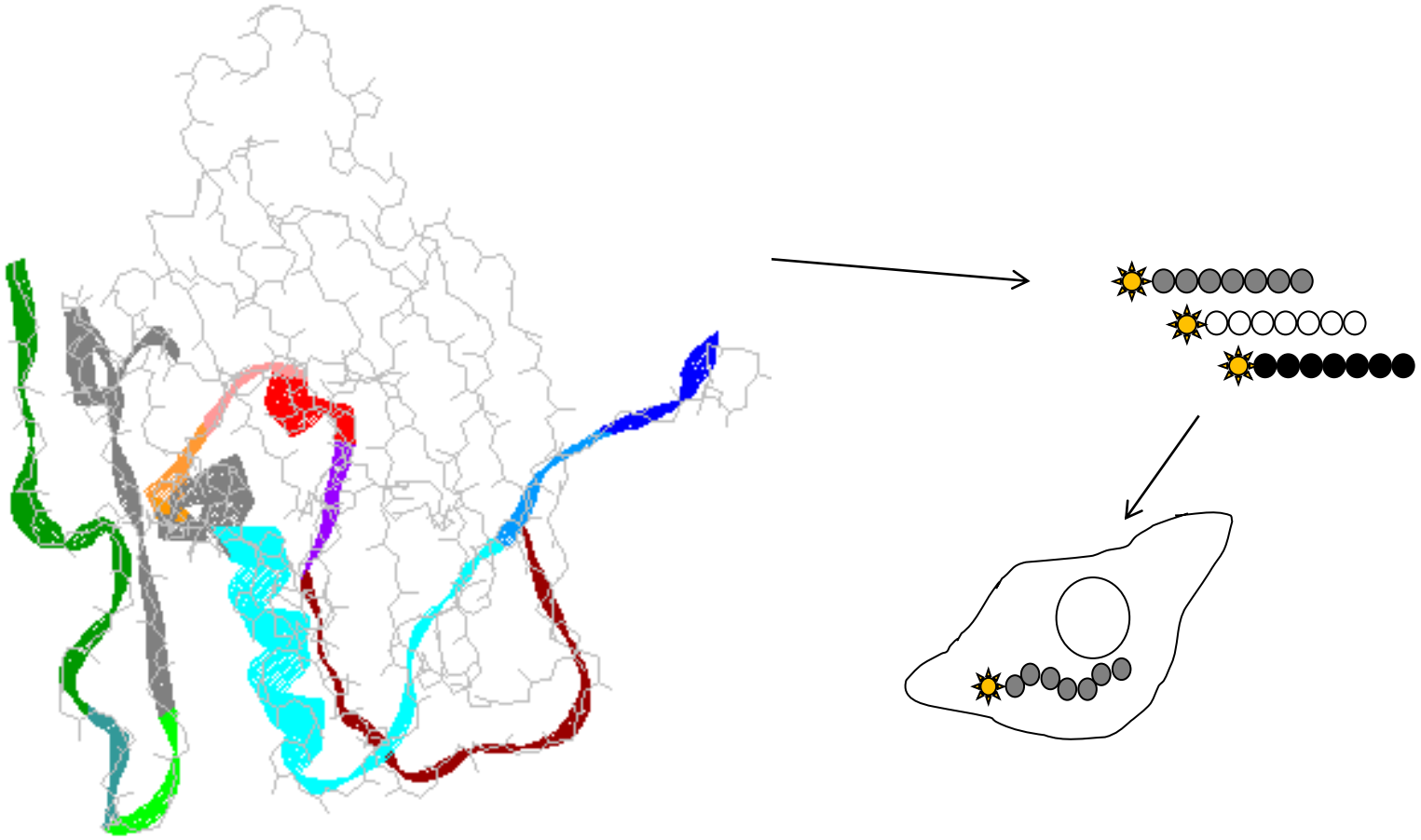
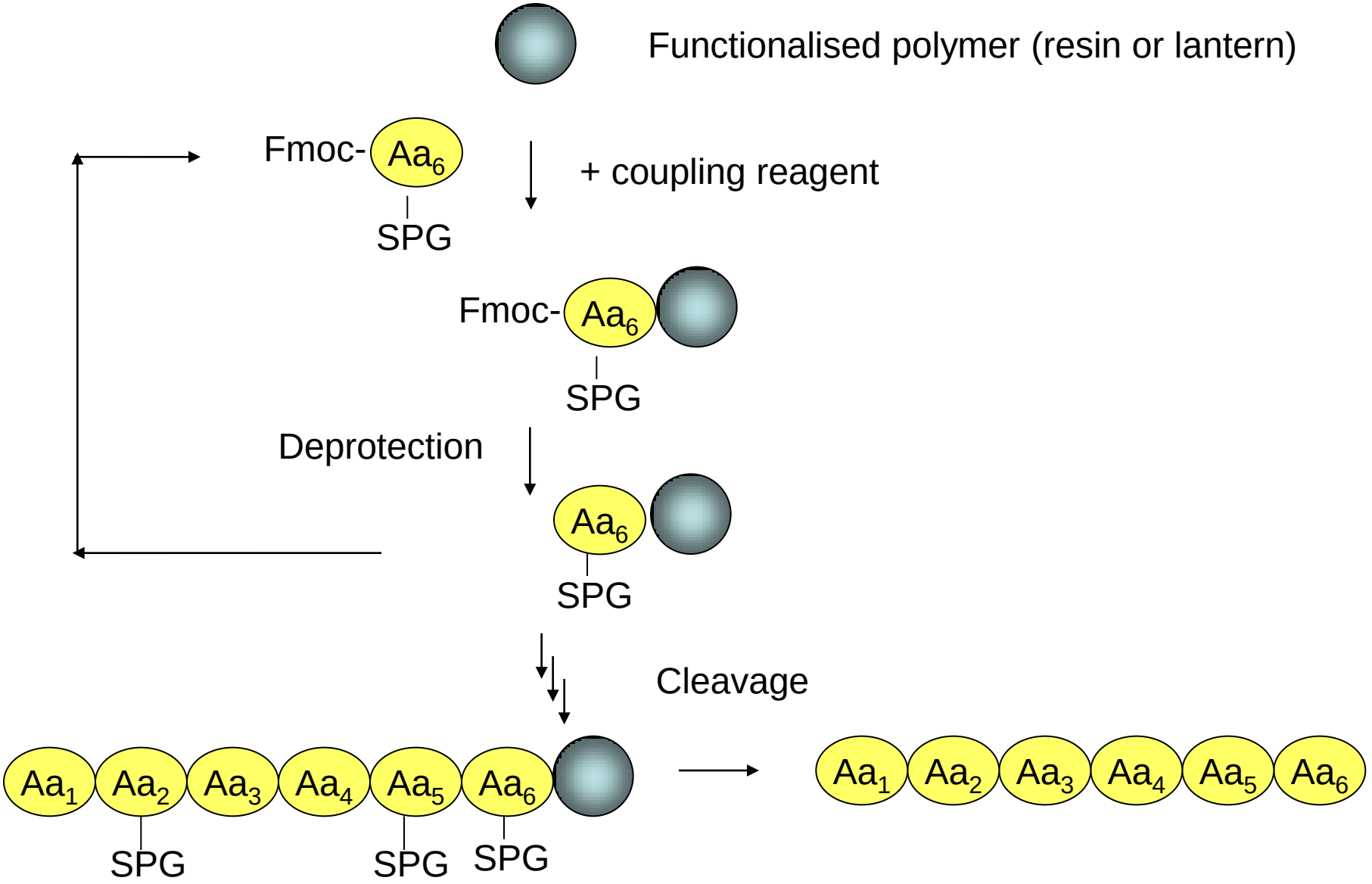
Di Giovine et al: PLoS Pathog 7(9):e1002277 (2011)

Strategy



^{21}HGVRGKYALADASLKMADPNRFRGKDLPVLDQLTDPPGVRRVYHIQA^{67}
^{206}LEHRAKGSCKYALPLRIPPSACLSPQAYQQGVTVDS^{241}
^{239}VDSIGMLPRFIPENQRTVAVYSLKIAGWHGPKAPYTSTLLPP^{280}

Solid phase peptide synthesis scheme



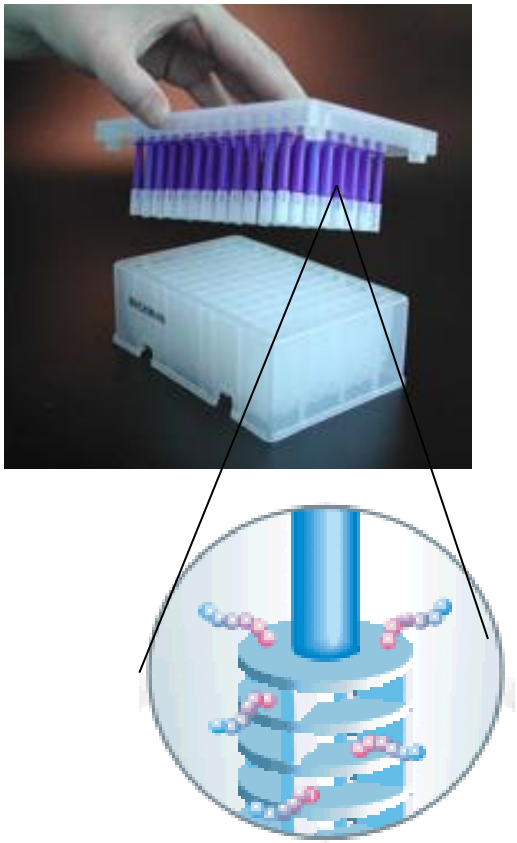
Peptide synthesis

Carrier: SynPhase ™ lantern (Mimotopes),
Capacity: 8 µmol
Fmoc/tBu strategy

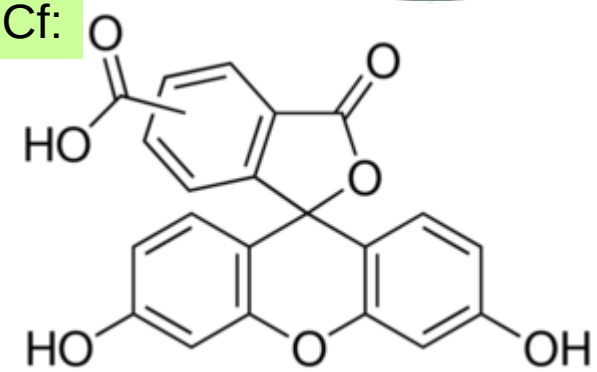
Fmoc cleavage: piperidine – DBU – DMF
(2:2:96 V/V/V)
DIC/HOBt coupling 2x, 10 eq
Monitoring: bromophenolblue

Labelling: 5(6)-carboxyfluorescein (Cf),
DIC/HOBt coupling

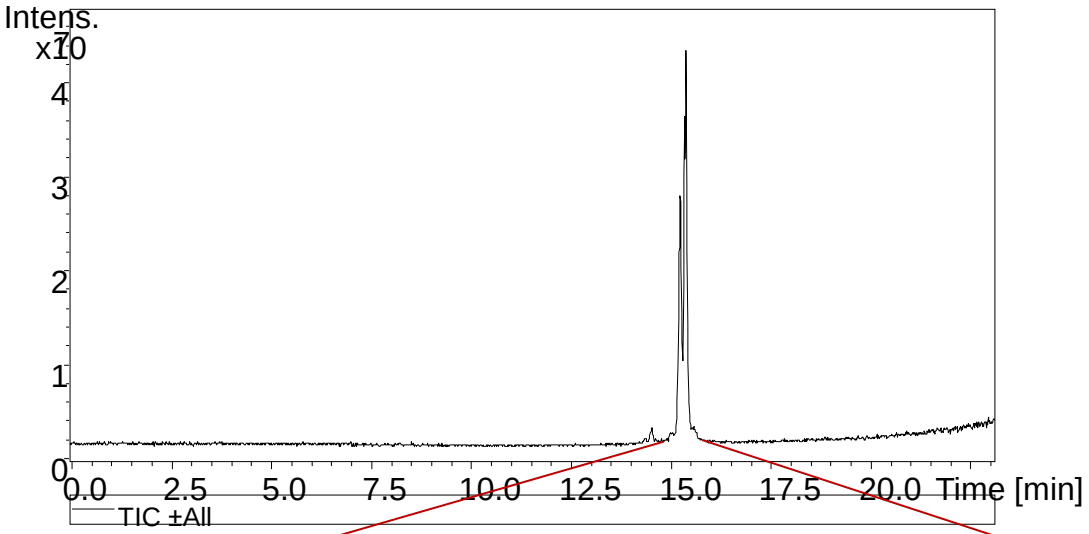
Cleavage: 1.5 h
TFA – thioanisol – water – phenol –
1,2-ethandithiol (80:5:5:7.5:2.5, V/V/V/m/V)



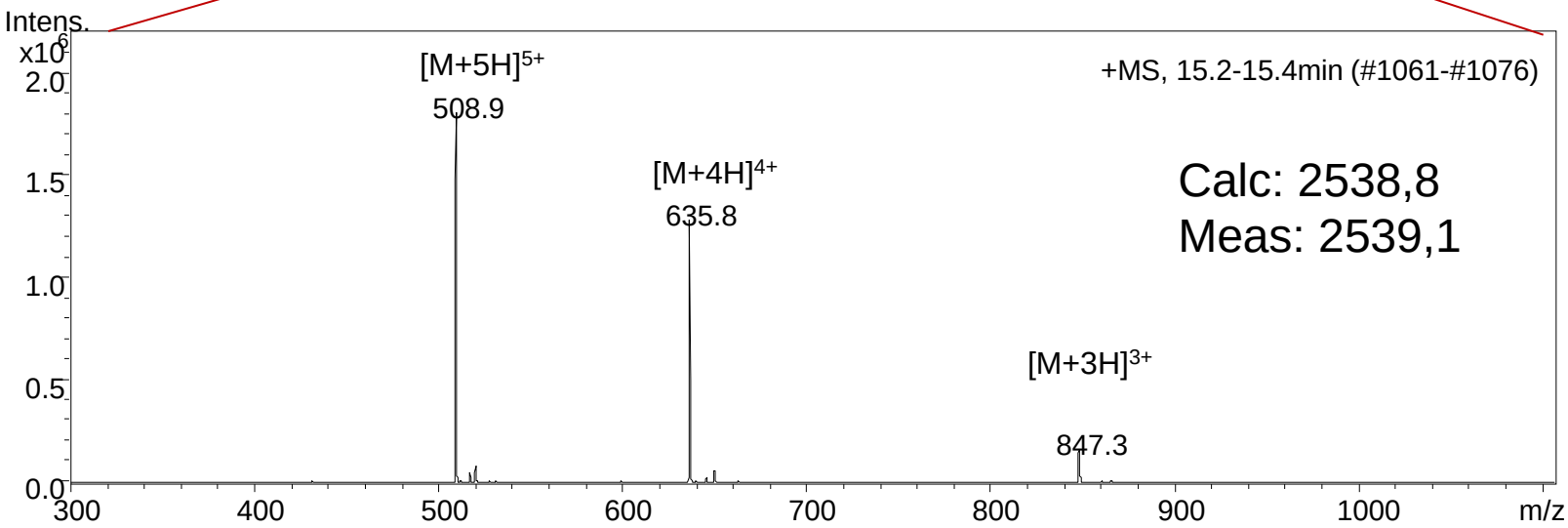
Cf:



HPLC-MS chromatogram of a purified Cf-HSV-gD peptide

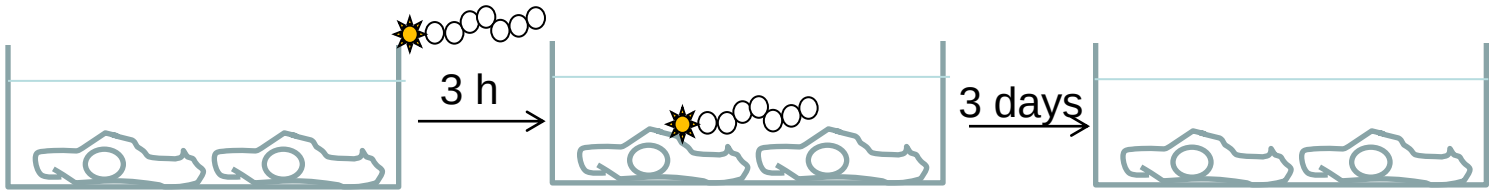


Jasco PU-2085 Plus Semi-Micro HPLC, Phenomenex Synergi C18, 100 x 2,0 mm, 2.5 µm, 100 Å



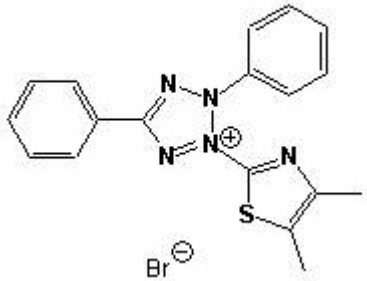
Bruker Daltonics Esquire 3000+

In vitro cytostatic effect of Cf-HSV peptides, MTT assay

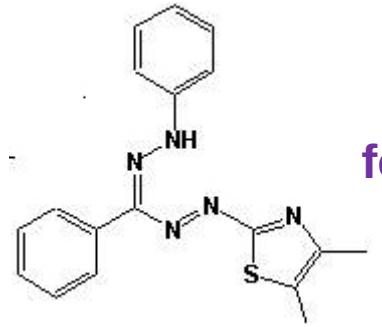


3-5x10^3 SH-SY5Y neuroblastoma cells

MTT



Mitochondrial succinate dehydrogenase



formazane

Live cells
Intact mitochondrium

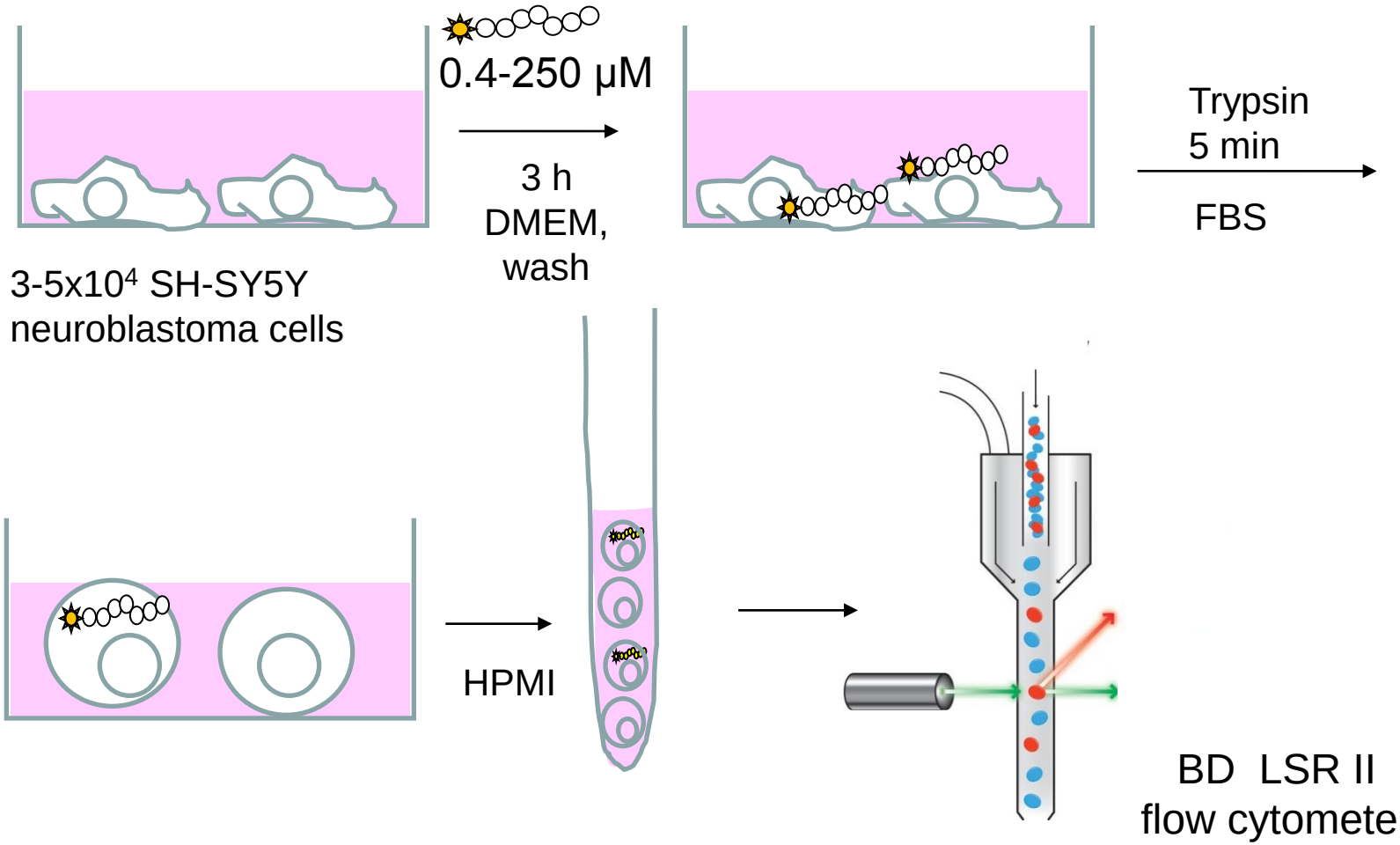
Dissolving in DMSO →

OD: λ_1=540 nm; ref: λ_2=620 nm

MTT: 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide

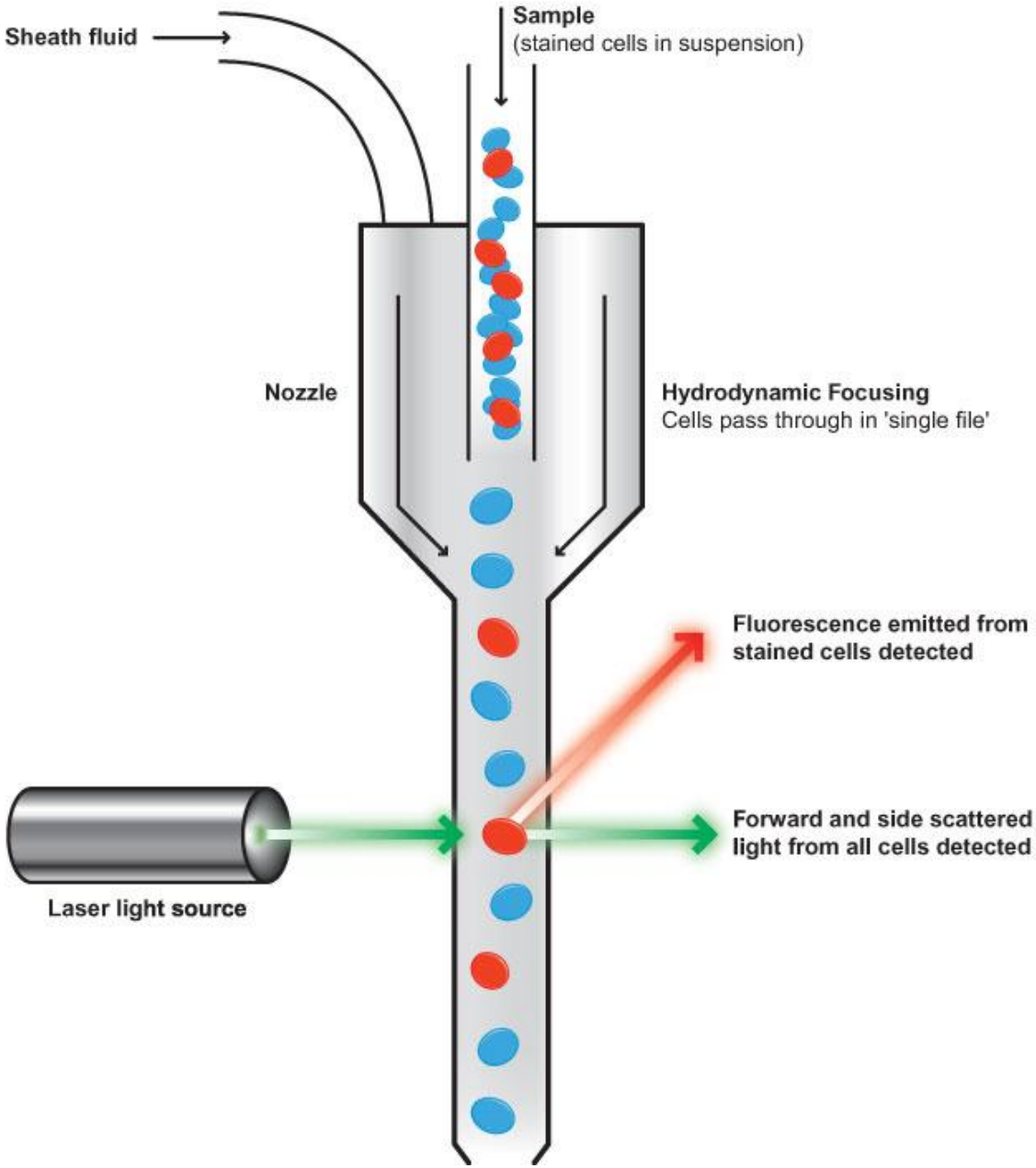
The peptides are not cytostatic in the conditions to be used for cellular uptake experiments

Studying the *in vitro* celllular uptake of Cf-HSV peptides, flow cytometry



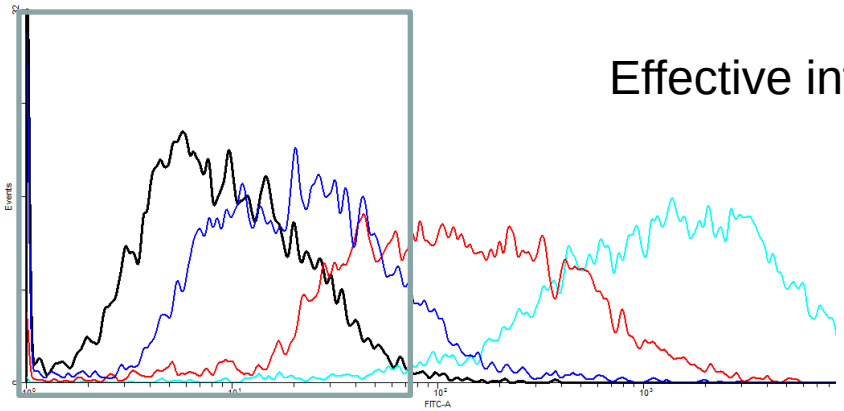
Coherent Sapphire laser, 22 mW, λ_{exc}= 488 nm
FITC, LP 510, BP 530/30 (PE, LP 550, BP 576/26)

Flow Cytometry



Internalisation of Cf-HSV peptide into SH-SY5Y cells

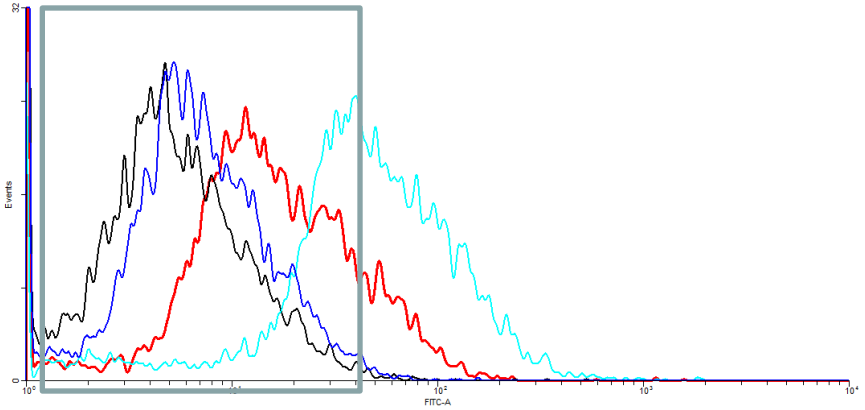
Number of cells

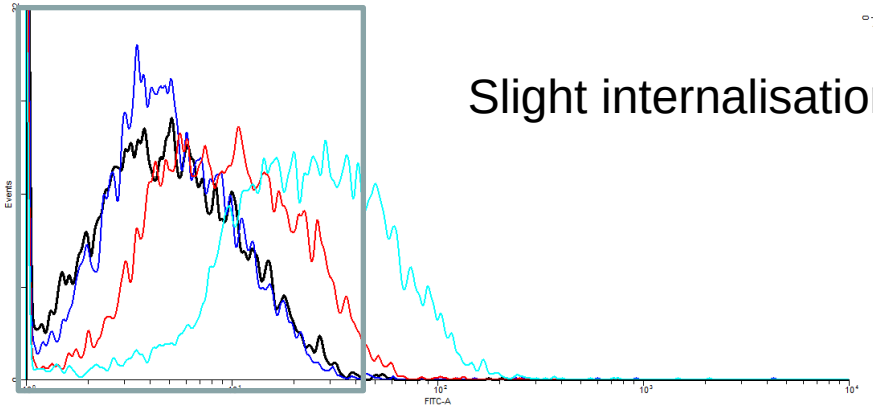


Effective internalisation

50 µM
10 µM
2 µM
kontroll

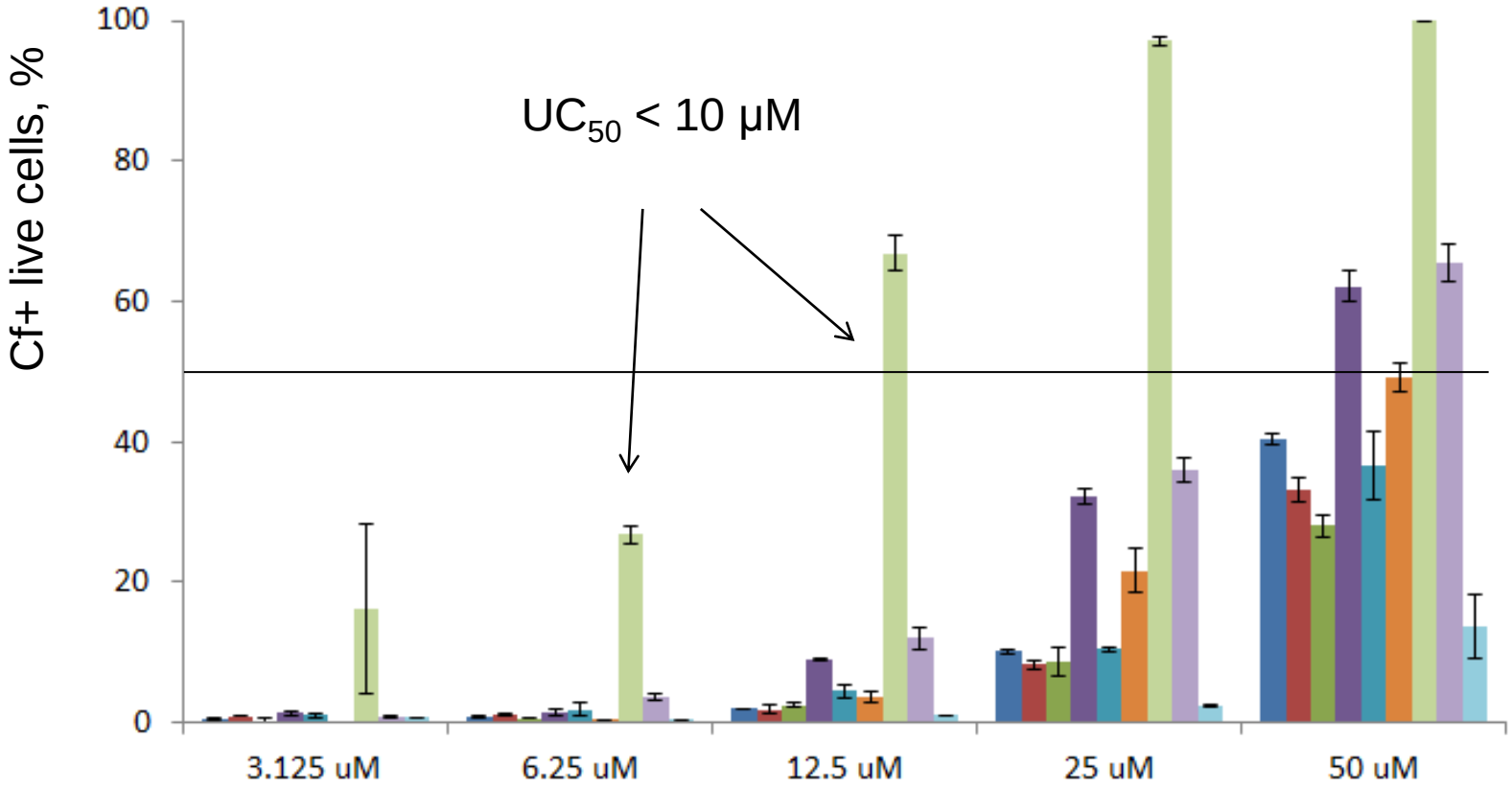
Fluorescence intensity





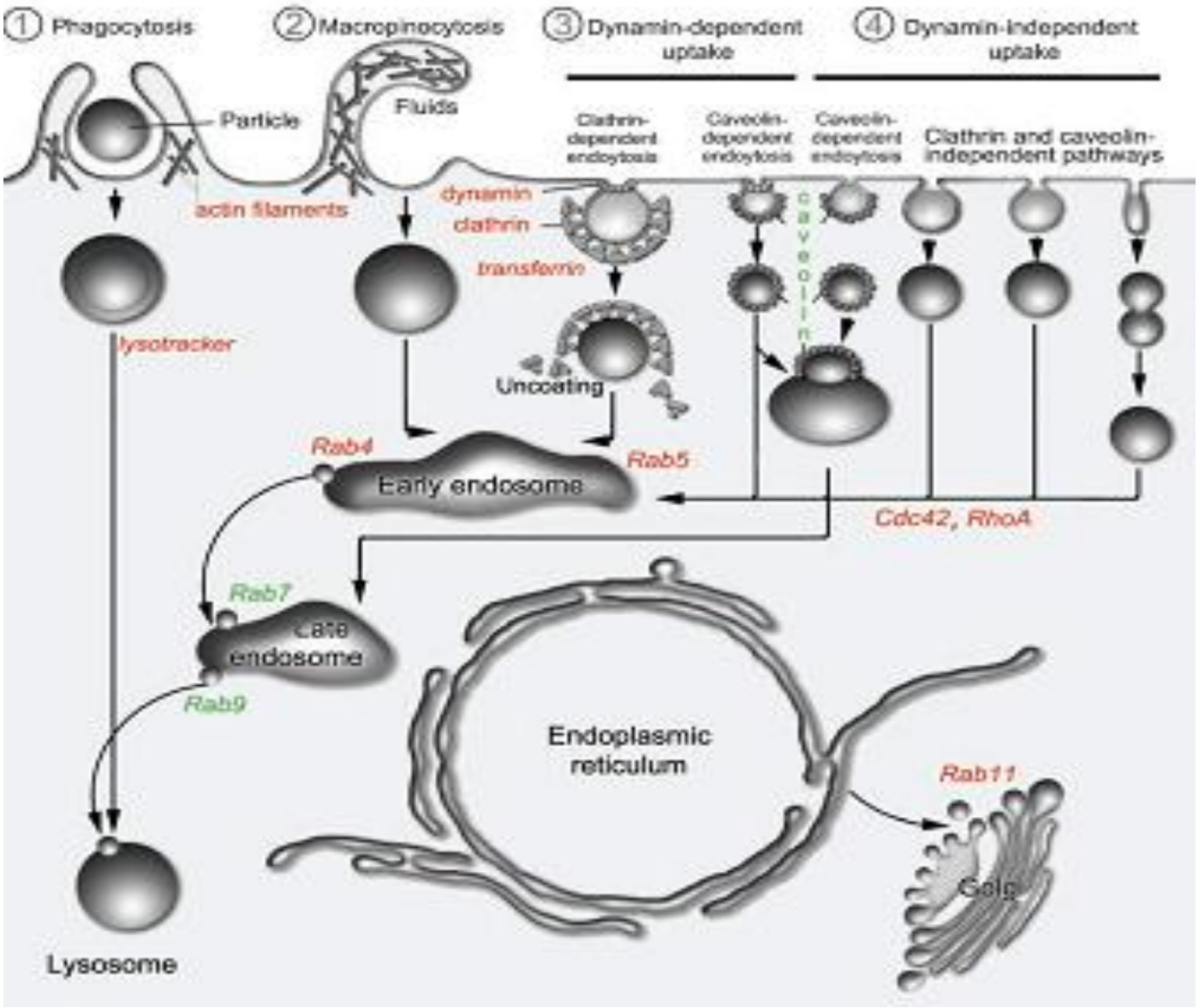
Slight internalisation

Internalisation of Cf-HSV peptides into SH-SY5Y neuroblastoma cell

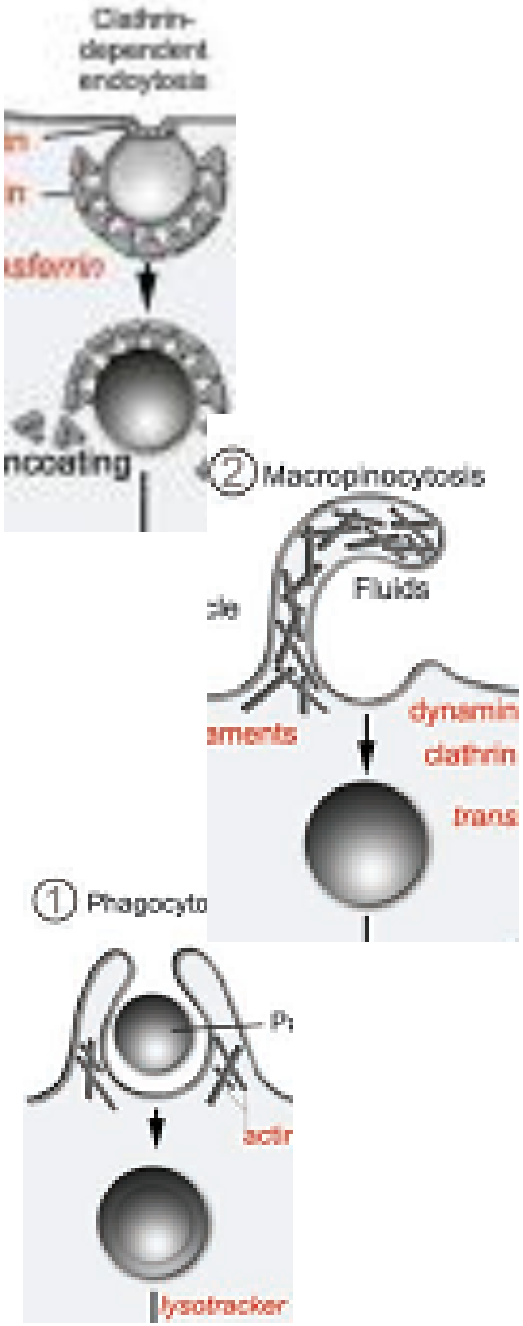


UC_{50}: 50% uptake concentration – 50% of the cells contains Cf-labelled peptides

Endocytosis pathways



Flynn et al: J Pharm Sci. ;99(7):3100-3121. (2010)

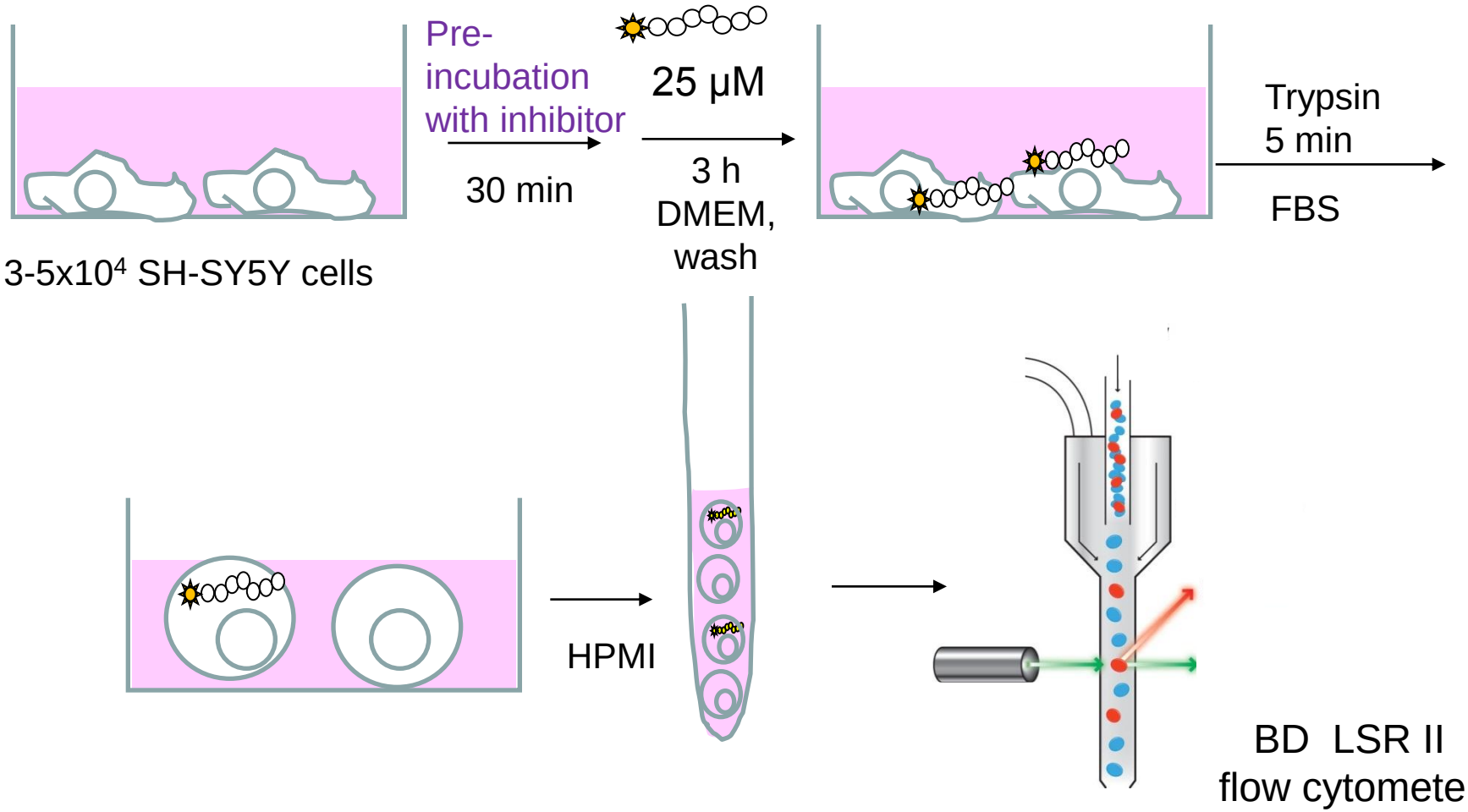


Inhibition of cellular uptake

Inhibitor	Mechanism	Type of endocytosis
Cytochalasin D	Actin polymerisation inhibitor	Macropinocytosis, chlatrin dependent endocytosis[1],[2]
EIPA (5-(*N*-ethyl-*N*-isopropyl)amylorid)	Selective Na^+/H^+ antiport inhibitor	Macropinocytosis[2,3]
Colchicin	Microtubule polymerisation inhibitors	Pinocytosis[4]
Methyl-β-cyclodextrin	Cholesterol depletion from membrane	Caveola/lipid raft mediated endocytosis[5]

[1]Nakase et al, Mol. Ther. (2004) 10: 1011-1022
[2]Delwig et al, Arthr. Res Ther. (2006) 312: 1345-1360
[3]Heikkilä et al, J. Virol. (2010) 84: 3666-3681
[4]Piasek et al, hematol. Blood Transf. (1985) 29: 511-513
[5]Rodal et al, Mol. Biol. Cell (1999) 10: 961-974

Inhibition of the *in vitro* celllular uptake of Cf-HSV/253-272, flow cytometry

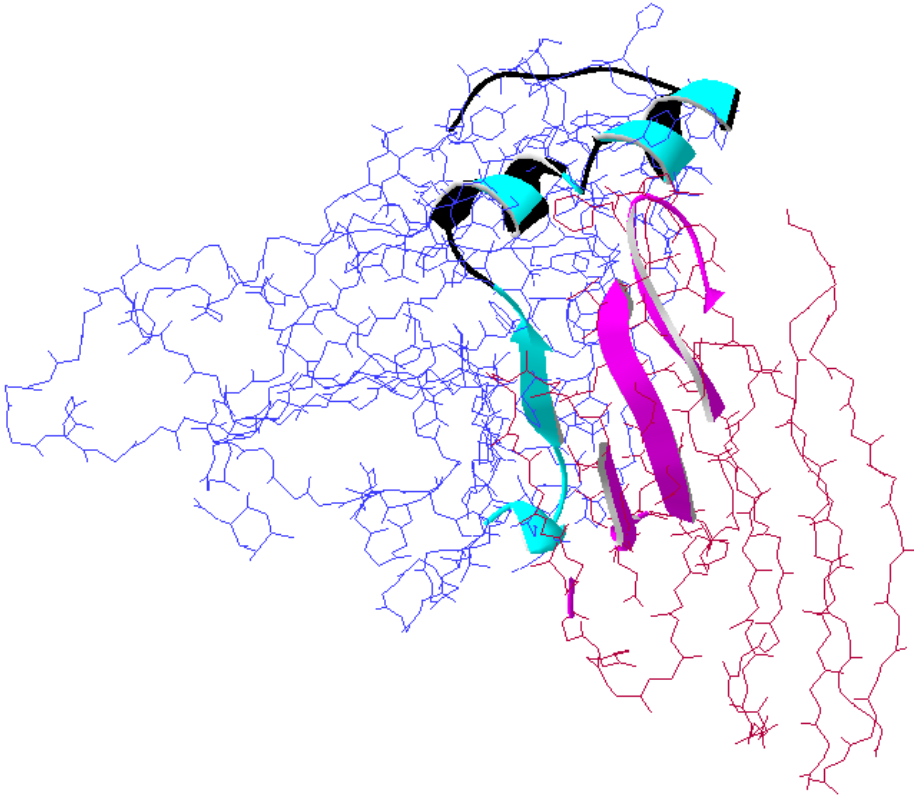


Coherent Sapphire laser, 22 mW, λ_{exc}= 488 nm
FITC, LP 510, BP 530/30 (PE, LP 550, BP 576/26)

Summary

Cf-labeled HSV-1 gD 20-mer peptides internalised into SH-SY5Y neuroblastoma cells
at different rates,
in concentration dependent manner.
Few nectin-binding peptides internalised with high effectivity, even in small concenration

Uptake of peptide was inhibited by EIPA
=> Method of entry:
macropinocytosis – compatible with receptor mediated endocytosis



Further research needed:

Specificity	uptake on other cell lines
Method of entry	inhibition studies on more peptides
Exact sequence requirements	shorter, modified peptides, changing oxidable amino acids

Conjugation of drugs for targeted herpes or tumour therapy

Acknowledgements

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Thank you for your attention!