DESIGNING EFFECTIVE ANTICANCER-RADIOPEPTIDE CARRIERS: A MOLECULAR DYNAMICS STUDY OF THEIR INTERACTION WITH MODEL TUMOUR AND HEALTHY CELL MEMBRANES

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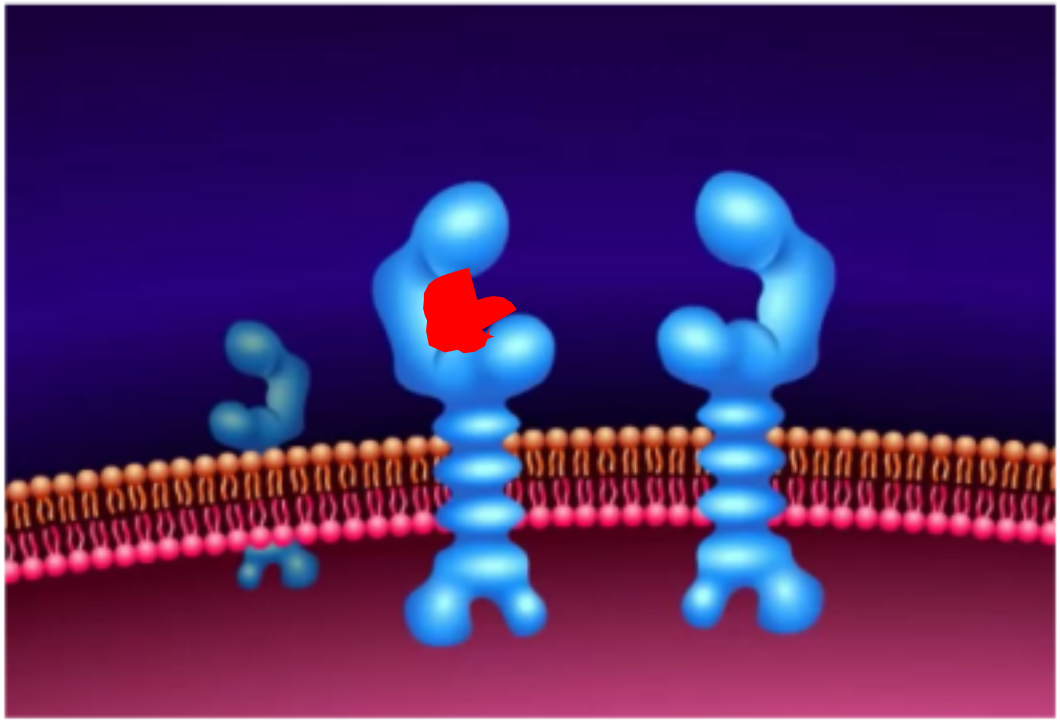
SUMMARY

- Peptides in oncology: a possible therapeutic strategy
  - Design of a therapeutic radio peptide
    - Molecular dynamics:
      peptide - (tumour/healthy) membrane interactions
      - Conclusions & Outlook

PEPTIDES IN ONCOLOGY

(some) cancer cells overexpress membrane receptors

Properly shaped, drug-carrying peptides can be designed to interact with these receptors and selectively kill cancer cells



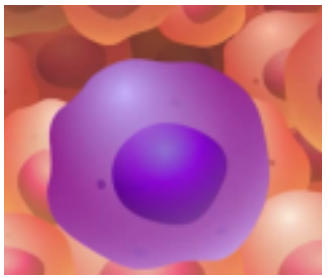
Drawbacks:

1. Not all cancer cells overexpress receptors
2. Some receptors are present also in healthy cells
3. A specific peptide must be designed for each cancer

PEPTIDES IN ONCOLOGY

An alternative approach: design a peptide interacting with various tumour cells, exploiting features common to cells of different tumours.

Tumour cell membranes are **negatively charged** & exhibit an extracellular **acidic pH**.

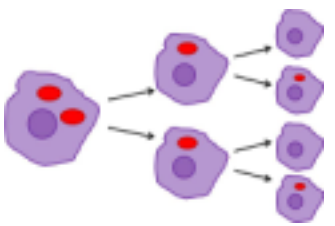


Increased glycolysis
High lactate levels
Lactate binds cations and moves them away

A peptide fulfilling 2 requirements

a - neutral at physiological pH
b - positively charged at acidic pH

could be selective for tumour cells



pH < 7,2
Lactic acid is in excess because tumour cells don't have enough mitochondria and exploit fermentation to produce ATP

Chen *et al.* Theranostic 2016

ANTIMICROBIAL PEPTIDES

A GOOD STARTING MODEL

LL37, an antimicrobial peptide, is taken as a starting model

LL-37

- Wide spectrum antimicrobial activity
- 37 amminoacids
- +6 charge at physiological pH
- α helix conformation

NH_2-LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES-COOH

THE MODIFIED PEPTIDE

Modifications to optimize the peptide for tumour membrane binding:

1- LL-37 binds a sugar present in epithelial cells

To prevent it from sticking to healthy cells the binding region is cut

2- Some positive aas → HIS residues

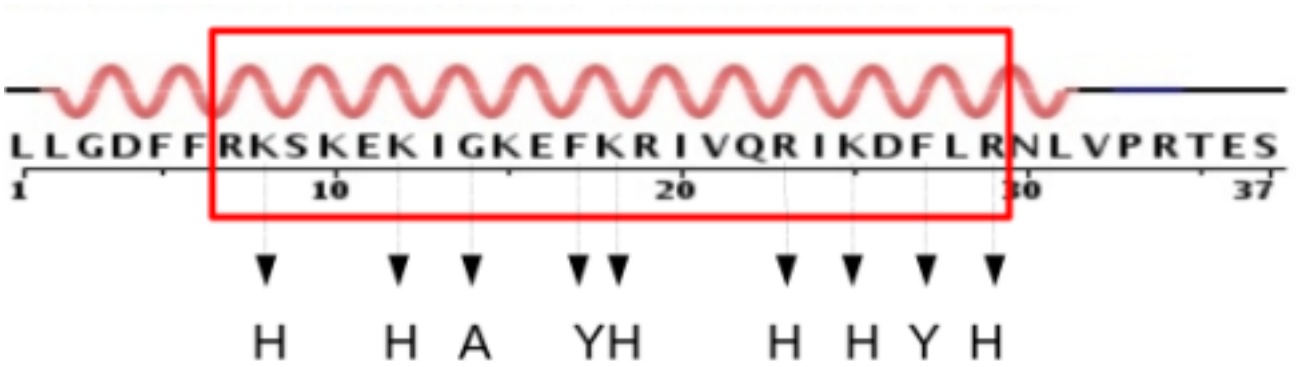
HIS becomes positively charged at acidic pH

3- PHE → TYR

Increased solubility at physiological pH of the neutral peptide

THE MODIFIED PEPTIDE

The new peptide: RH-23



NH_2-RHSKEHIAKEYHRIVQHIHDYLH-COOH

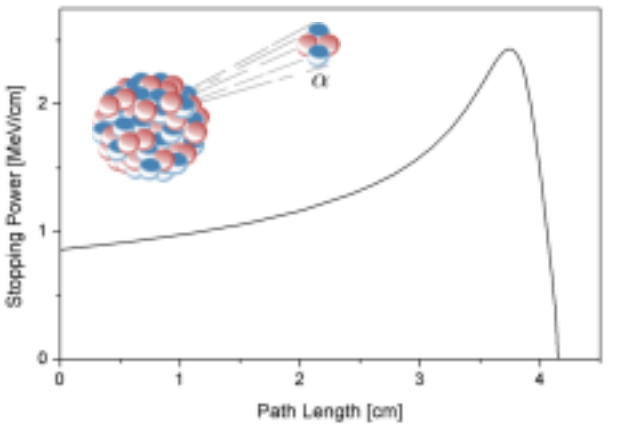
It fulfils the 2 required conditions:

(a) **neutral** at **physiological pH**

(b) **positively charged** at **acidic pH**

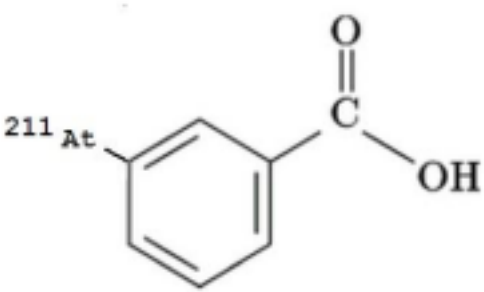
RADIO LABELLING

An α-emitter is the active element bound to RH-23



A good choice is ^{211}At

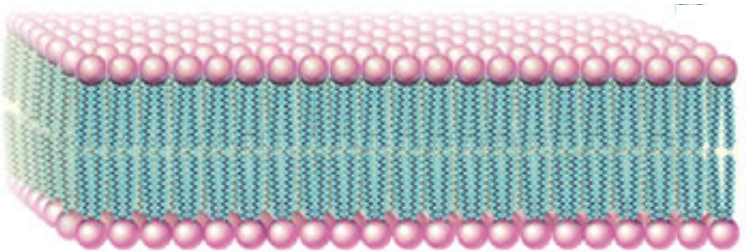
In MD simulations we model it as ^{80}Br,
since At and Br have similar valence and VdW radius

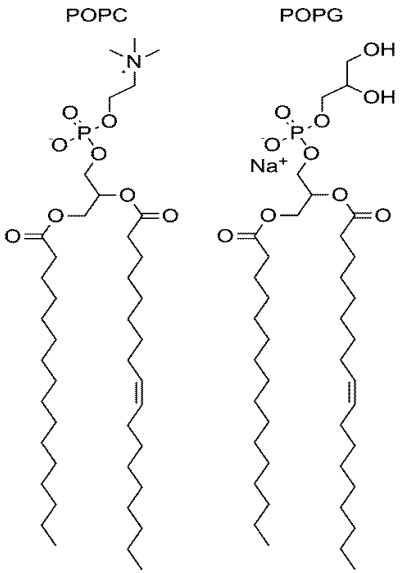


The heavy Br atom is bound to the peptide via a benzoic acid

HEALTHY AND TUMOUR MEMBRANES

Both membranes are modelled as a lipid bilayer





Neutral, healthy membrane: 100% POPC lipids

Charged, tumour membrane: 70% POPC + 30% POPG

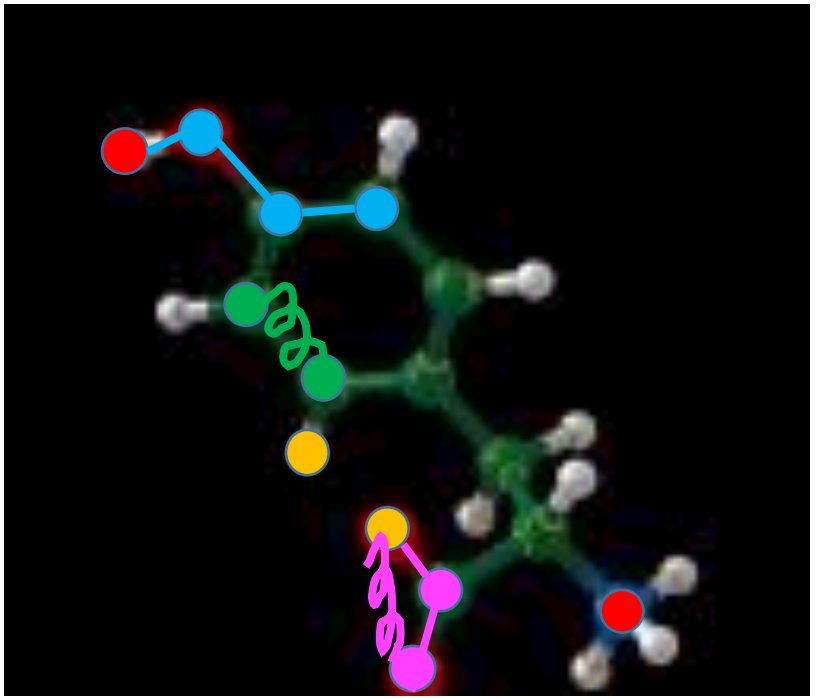
POPC: Palmitoyl-Oleoyl-Phosphatidylcholine

POPG: Palmitoyl-Oleoyl-Phosphatidylglycerol

MOLECULAR DYNAMICS

Simplest form

Atoms like spheres & bonds like springs



Potential Energy =

Bond STRETCHING energy

+ Angle BENDING energy

+ Dihedral TORSION energy

+ NON-BONDED interaction energy

(van der Waals + Coulomb)

Equation form + Parameters = Force Field

MOLECULAR DYNAMICS

4 different systems are simulated
NpT ensemble @ 310 K

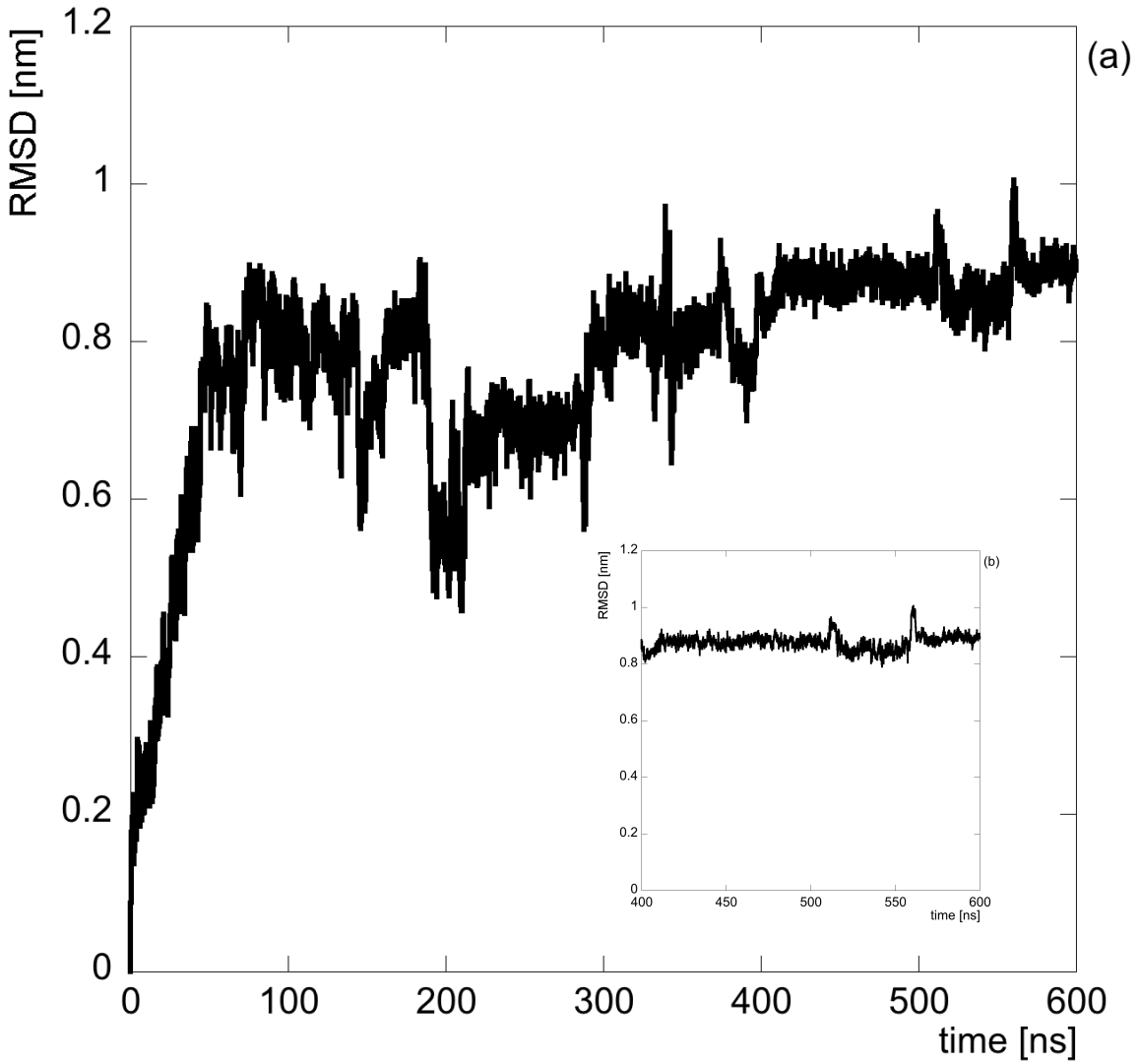
NAME	SYSTEM COMPOSITION	LENGTH	# of TRAJ
nRH-23w	^{Br}RH-23$_0$ + water	600 ns	1
nRH-23h	^{Br}RH-23$_0$ + 144 POPC per layer + water	200 ns	9
pRH-23t	^{Br}RH-23$_+$ + 101 POPC & 43 POPG per layer + water	200 ns	9
pRH-23h	^{Br}RH-23$_+$ + 144 POPC per layer + water	200 ns	9

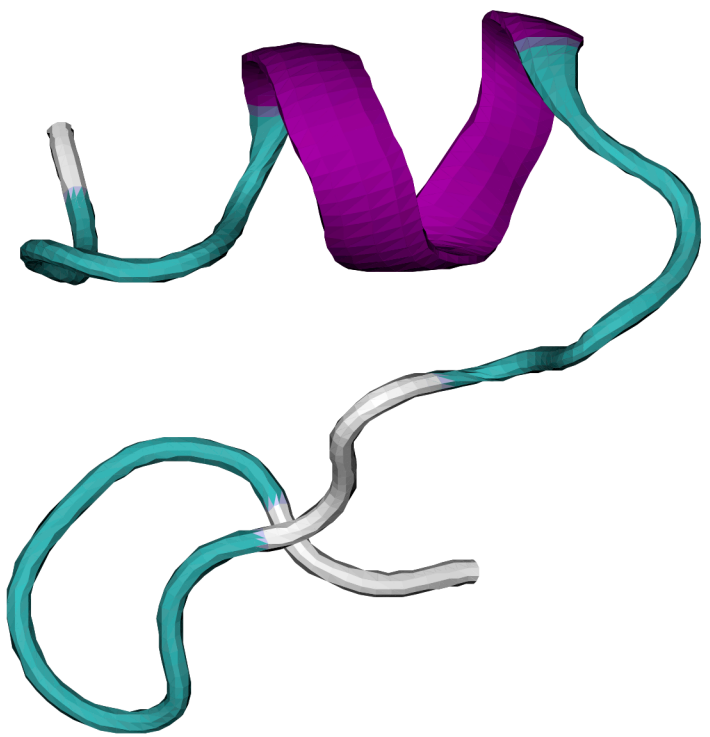
MD trajectories simulated using GROMACS

Some 10^5 core hours on CINECA
Marconi & Galileo clusters

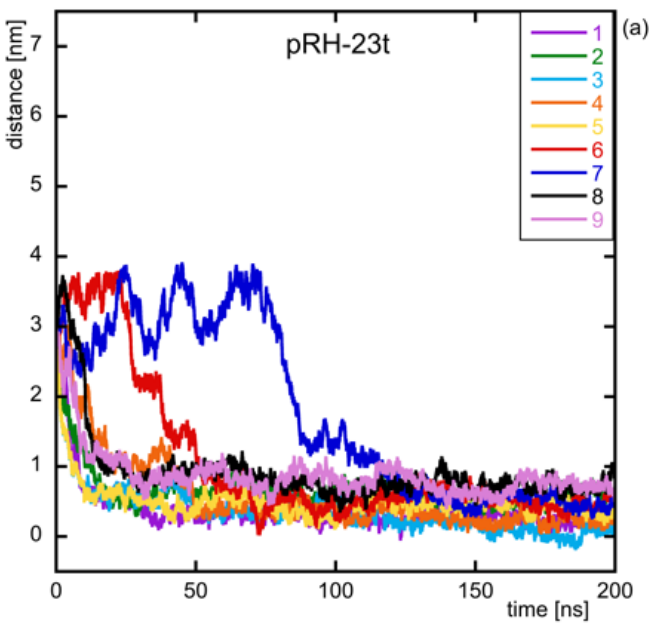
Capozzi *et al. (2017)* submitted JCTC

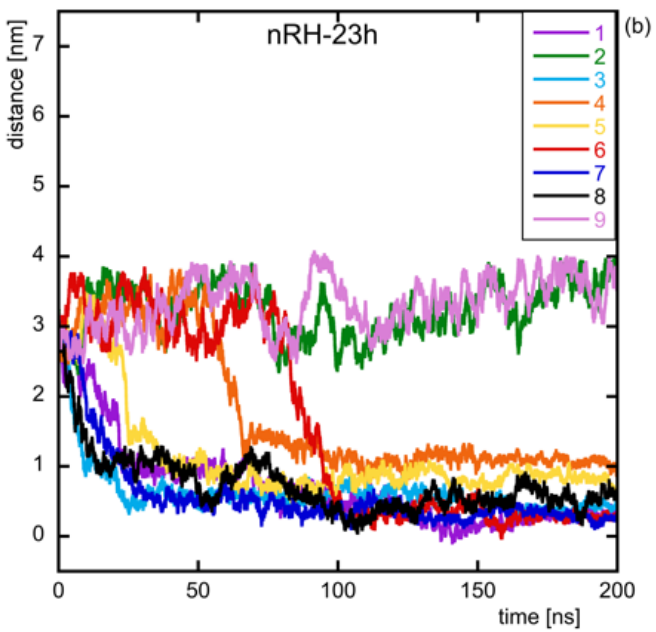
nRH-23w

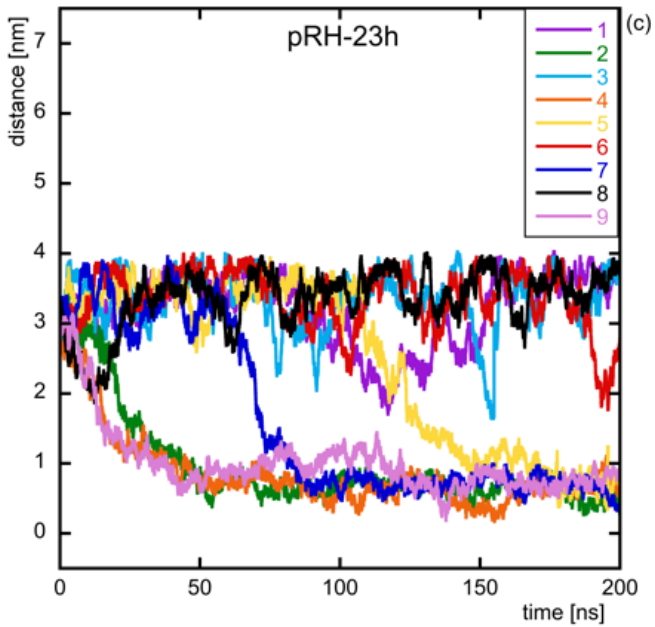

RMSD [nm]
1.2
1
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
600
time [ns]
(a)
RMSD [nm]
1.2
1
0.8
0.6
0.4
0.2
0
400
450
500
550
600
time [ns]
(b)



PEPTIDE-MEMBRANE DISTANCE







pRH-23t → in **9 cases over 9** the peptide **gets** to the membrane

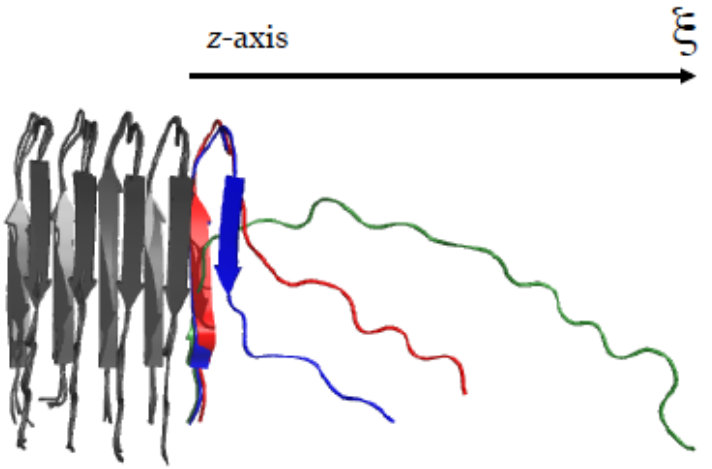
nRH-23h → in **7 cases over 9** the peptide **gets** to the membrane

pRH-23h → in **5 cases over 9** the peptide **gets** to the membrane

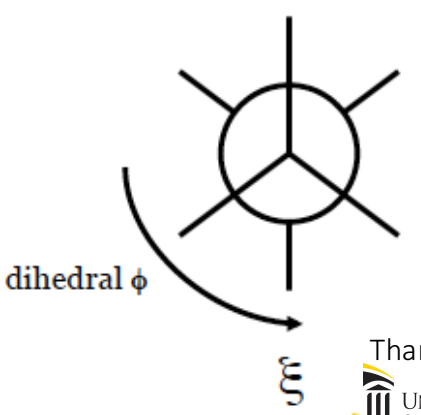
FREE ENERGY CALCULATIONS

Weighted Histogram Analysis Method (WHAM) in GROMACS.

- To calculate the potential of mean force (PMF), we define a reaction coordinate, ξ
  - A path along which the system evolves
  - Can be:
    - A direction (vector)
    - An angle (dihedral rotation)
    - Other abstract things



- Generate a series of configurations along ξ, conduct individual simulations at chosen intervals



Thanks to J. A. Lemkul

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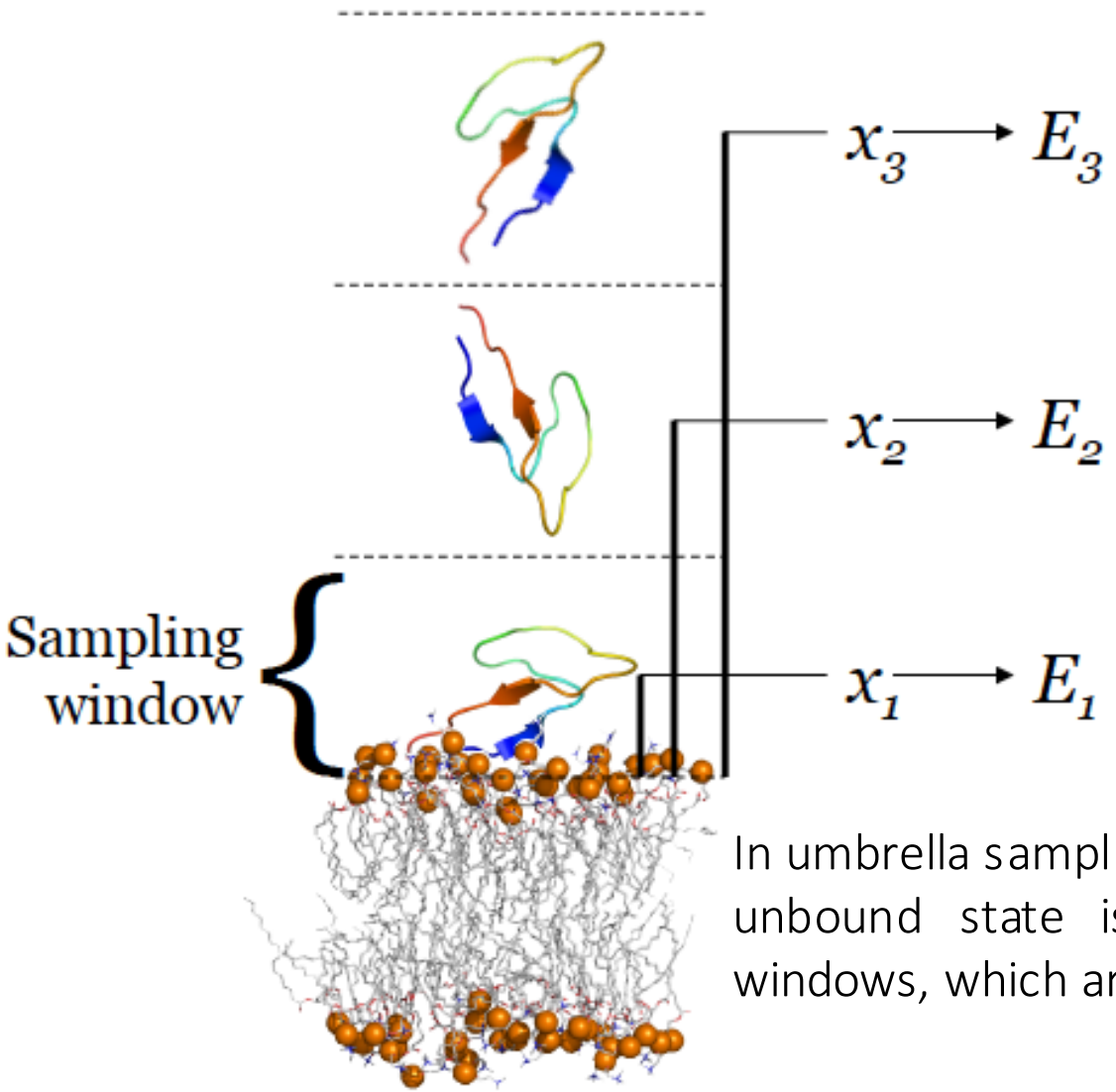
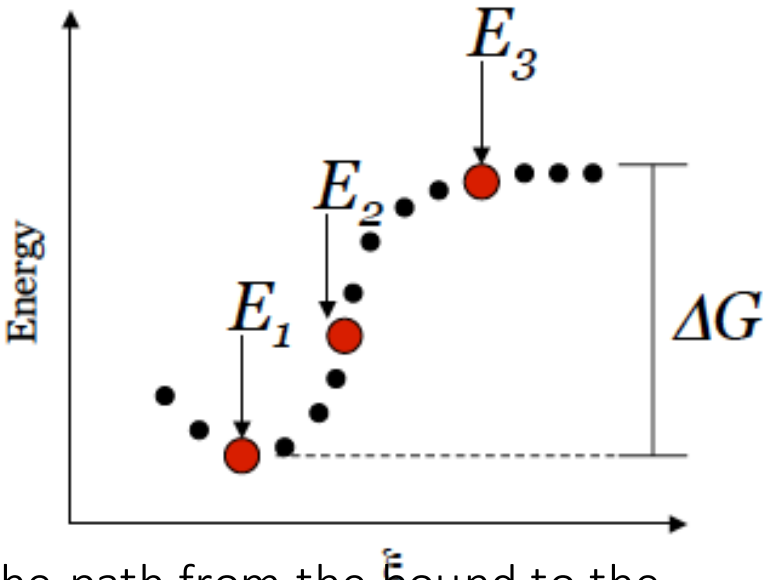
FREE ENERGY CALCULATIONS



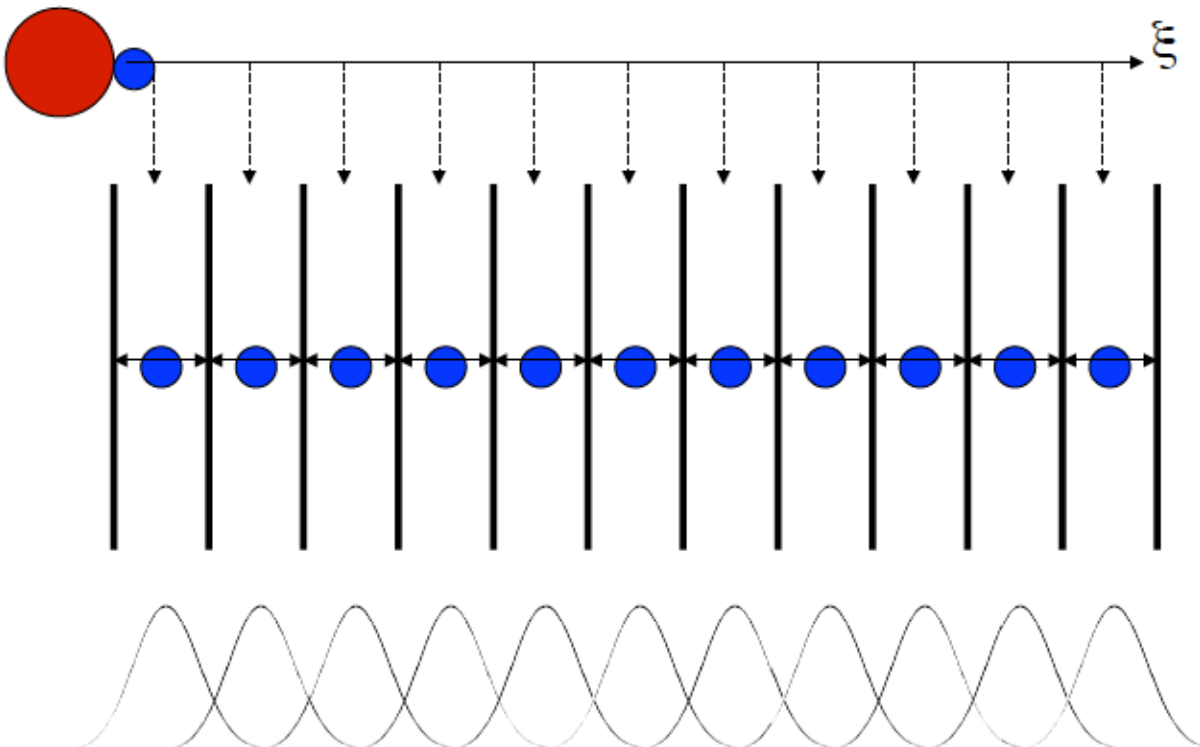


In umbrella sampling, the path from the bound to the unbound state is divided in a finite number of windows, which are independently sampled

Thanks to J. A. Lemkul

FREE ENERGY CALCULATIONS

Umbrella sampling extracts the PMF yielding the ΔG for the binding/unbinding process



Harmonic potential allows for oscillation within each window, overlap with neighboring sampling windows.

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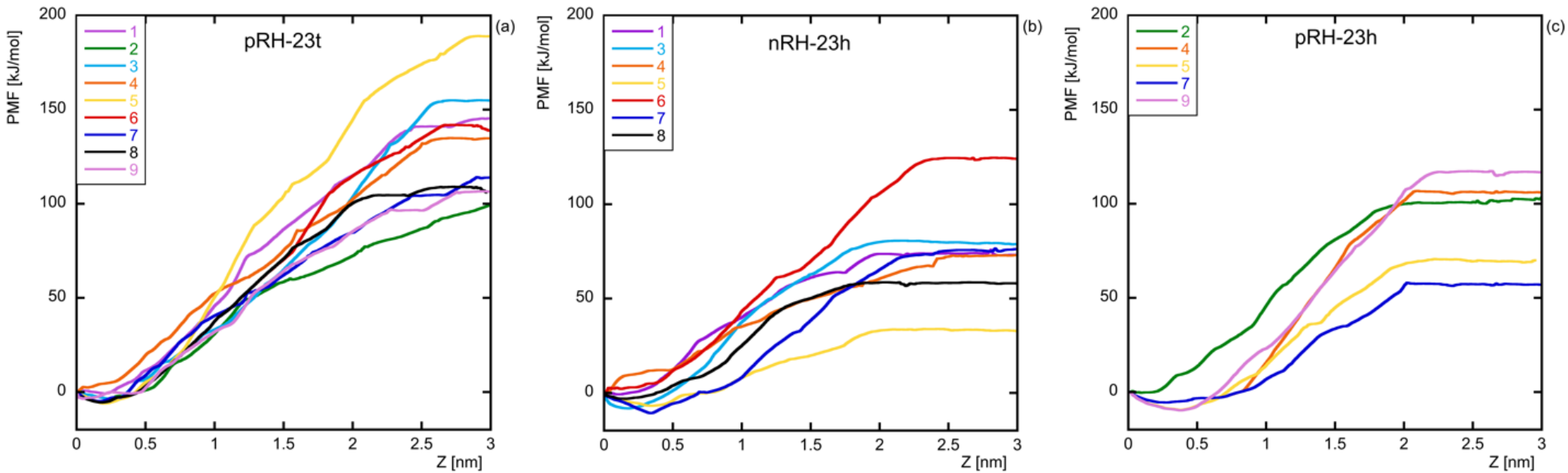
BINDING FREE-ENERGY



Free-energy differences are computed by pulling the peptide from the proximity of the membrane to 3 nm.

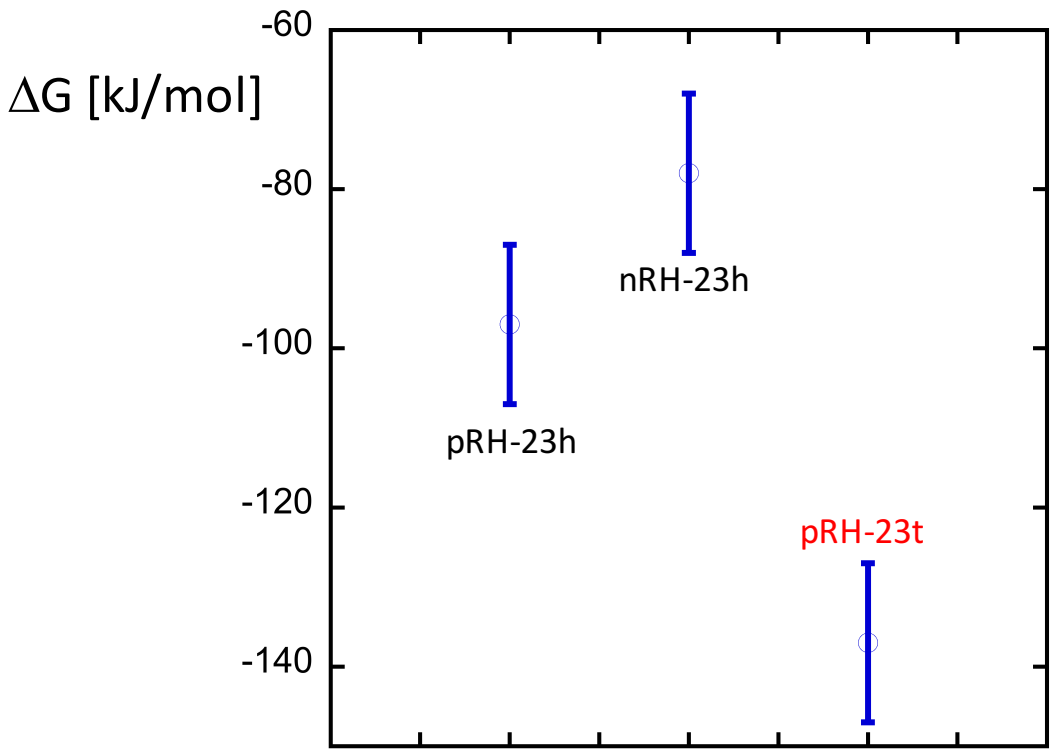
The PMF profile is obtained by recombining by means of the WHAM algorithm the energy data.

BINDING FREE-ENERGY

$\Delta G_{pRH\text{-}23h} = (-97 \pm 10)$ kJ/mol

$\Delta G_{nRH\text{-}23h} = (-78 \pm 10)$ kJ/mol

$\Delta G_{pRH\text{-}23t} = (-137 \pm 10)$ kJ/mol



CONCLUSIONS

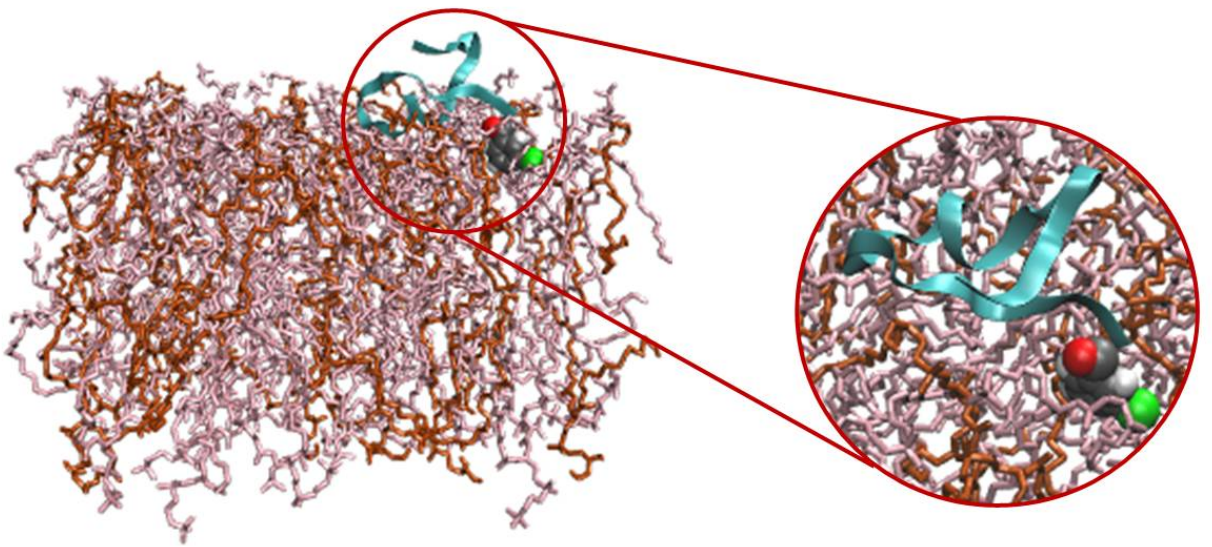
Peptides in oncology:

a really possible therapeutic strategy

pRH-23

binds tumour membranes

more tightly than healthy ones



OUTLOOKS

- Binding Iodine instead of Bromium
  → potential for tumour cell detection

- Perform *in vivo* experiments on animal models

BIOPHYSICS' GROUP IN TOR VERGATA

http://biophys.roma2.infn.it

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Giulia Romoli

Thank you for your attention!