Characterizing bradykinin-derived membrane-sensing peptides through Molecular Dynamics

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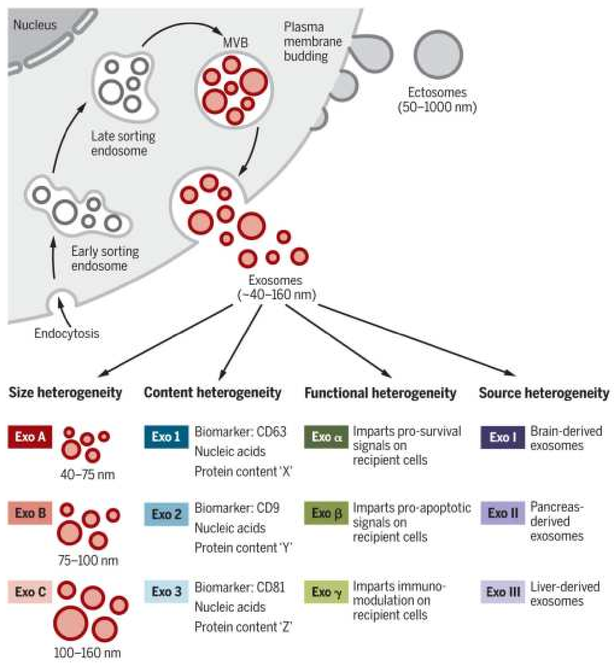
https://doi.org/10.17952/37EPS.2024.P2284

Finanziato dall'Unione europea
NextGenerationEU

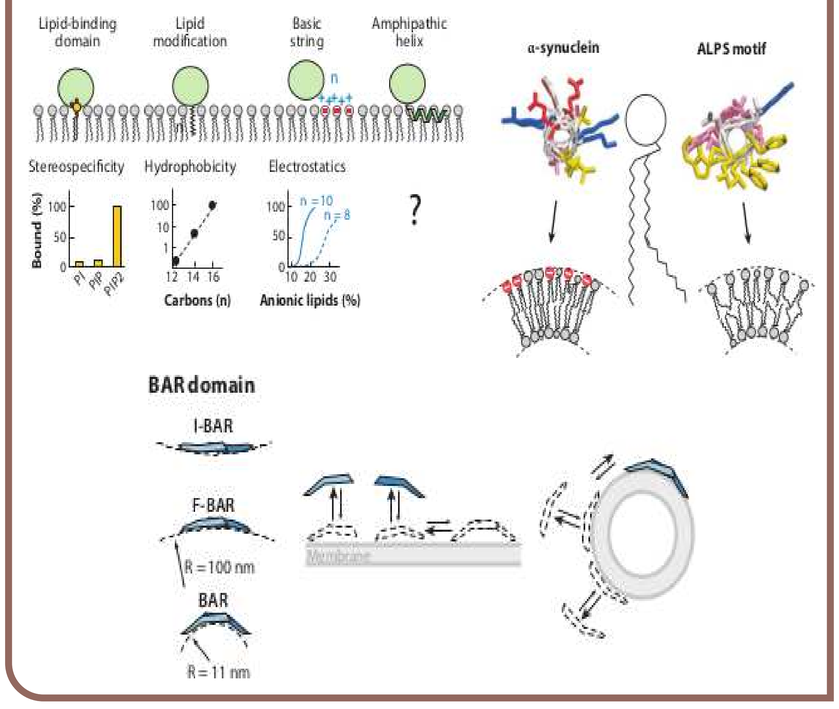
1. Abstract

Curvature-sensing peptides are peptides capable of recognizing high-curvature membranes like those of the Exosomes. Exosomes are extracellular vesicles that have raised a huge interest for their biomedical applications. The main challenge in the use of exosomes is the difficulty of purification by means of standard monoclonal antibodies. Alternative approaches, like those exploiting their membrane curvature are thus actively explored. In a recent work Cretich and coworkers tested three bradykinin-derived (BK) peptides (single, tandem and branched) showing that they recognize exosomes from the blood serum. NMR optimization simulations were first run on the BK-single peptide, using NMD-derived distance and torsion restraints. The parameters of a H-REMD simulation were tuned so as to reproduce the results of the NMR optimization, and the protocol was then used to study the conformations of the BK-tandem and BK-branched peptides. H-REMD simulations showed that the BK-single peptide adopts a W-shaped conformation that segregates hydrophobic and charged residues. While hydrophobic residues are mostly exposed on the convex side of the W-motif and might fit into the crevices of the membrane, charged residues are on the opposite side of the molecule. This arrangement is reminiscent to that of alpha-synuclein but BK-peptides realize residue segregation employing a structural motif alternative to the amphypathic helix of alpha-synuclein and ALPS motifs.

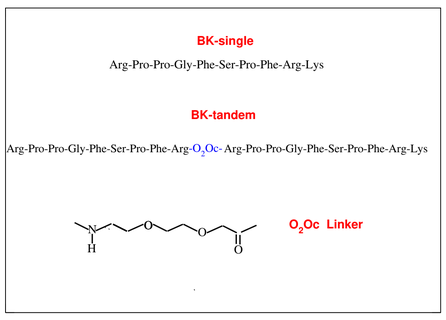
2. The Exosomes

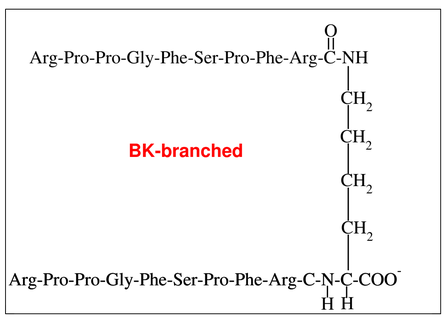


3. Curvature sensing



4. BK peptides: structure





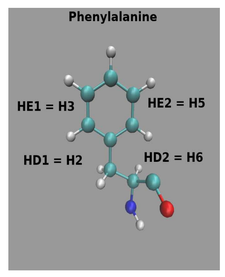
5. BK peptides: properties

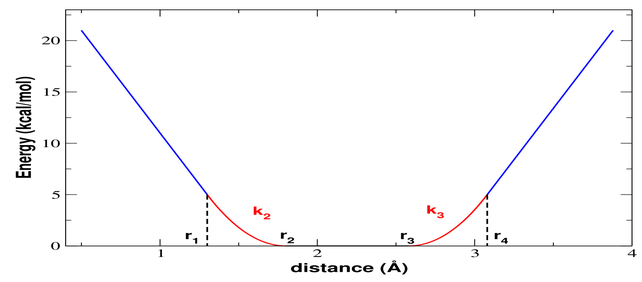
- Three variants of BK peptides:
  - BK-single decapeptide
  - BK-tandem peptide: two BK-decapeptides connected by a PEG linker O_2Occ
  - BK-branched peptide: Lys10, uses both the α and ϵ amine groups to bind two peptide chains
- BK-peptides interact with small (150 nm) but not large ($\leq$450 nm) liposomes
- BK-peptides successfull in purification of exosomes from blood serum.
- Exosome purification successfull even after protein digestion with trypsin.
- Multivalent peptides more effective than monovalent one in exosome but not in liposome recognition.

6. NMR optimization

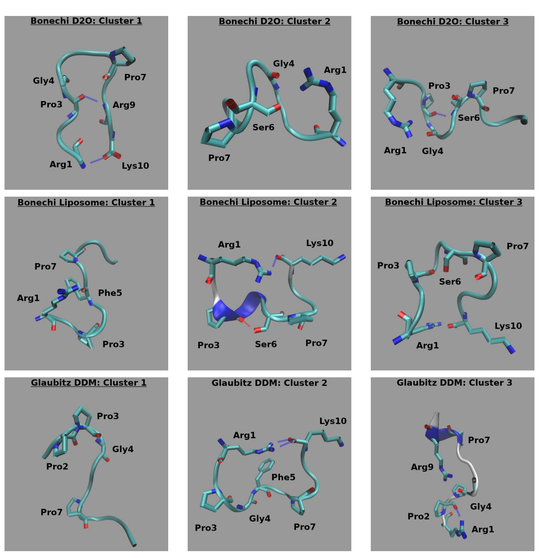
Intensity of cross-peaks from NOESY spectrum

Res1	Atom1	Res2	Atom2	Intensity	Dist (Å)
Pro3	Hδ 2	Pro2	Hα	Strong	2.4
Phe5	Hβ	Phe5	H(2,6)	Strong	2.5
Phe8	Hα	Phe8	H(3,5)	Medium	3.0
Phe8	H(2,6)	Pro7	Hβ1	Weak	4.0
Phe8	H(3,5)	Pro7	Hβ1	Weak	4.1
Phe5	Hβ	Phe5	H(3,5)	Weak	4.3
Phe8	Hβ1	Phe8	H(3,5)	Weak	4.6
Phe8	Hβ2	Phe8	H(3,5)	Weak	4.6





7. Annealing structures



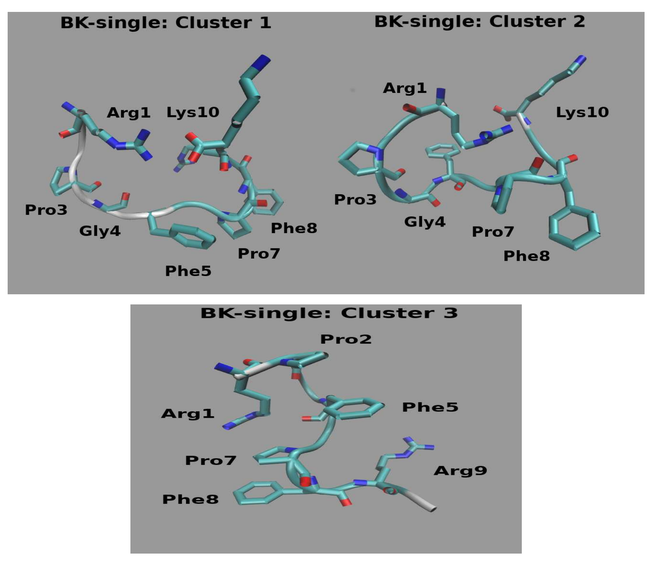
8. The REMD method

- Replicas are simulated in parallel at different temperatures with exchanges accepted based on Metropolis criterium.

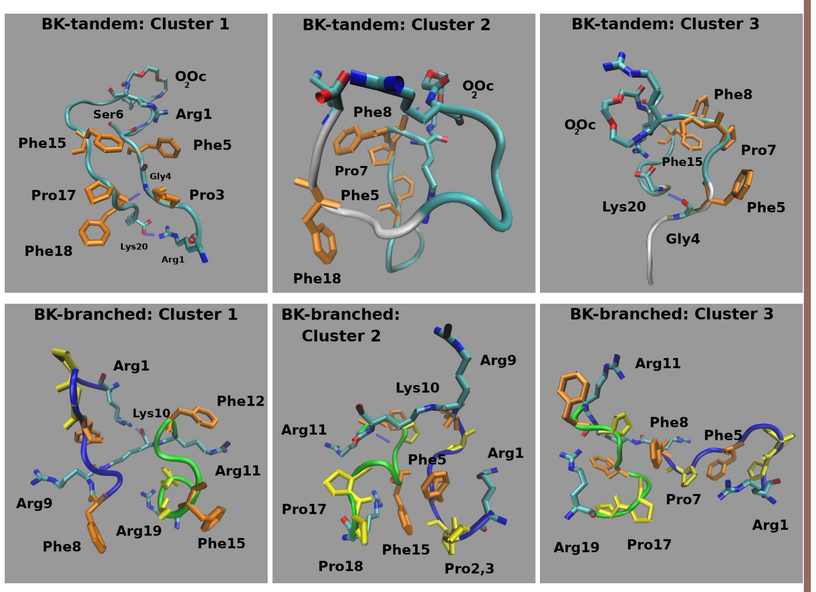
$$\begin{matrix}(T_1,\ldots,T_i,T_j,\ldots,T_N)\\(q_1,\ldots,q_m,q_n,\ldots,q_N)\\\Downarrow\\(T_1,\ldots,T_j,T_i,\ldots,T_N)\\(q_1,\ldots,q_m,q_n,\ldots,q_N)\end{matrix} \qquad P=\min[1,e^{-(\beta_i-\beta_j)(E(q_n)-E(q_m))}]$$

- Poor scaling with system size: $p_{acc}\propto\exp(\Delta\beta\Delta E)$
- Large systems $\Longrightarrow$ Large ΔE $\Longrightarrow$ Small $\Delta\beta$
- Hamiltonian REMD:replicas simulated at the same temperature but with different potential energy functions
- p_{acc} becomes function of a small subset of variables

9. H-REMD structures: 1



10. H-REMD structures: 2



11. Acknowledgements

Financially supported by the European Union - Next Generation EU

Title:

The virtual EV (v-EV): A digital twin of extracellular vesicles for health and food

(P2022EKHKL)

12. Conclusions

- Exosomes are promising tools both in diagnostics and therapeutics.
- Curvature-sensing peptides can be used to purify exosomes.
- Bradykinin proved successfull in the recognition of exosomes and its efficacy is enhanced by multivalency.
- In pure water the prevalent NMR structure of BK-single is a hairpin. The structure shifts to S- and W-shaped motifs in the neighborhood of a lipid micelle.
- H-REMD simulations show that both BK-single and the tandem and branched constructs adopt a conformation with segregation of a hydrophobic and a basic face similarly to α-synuclein and ALPS motifs.