

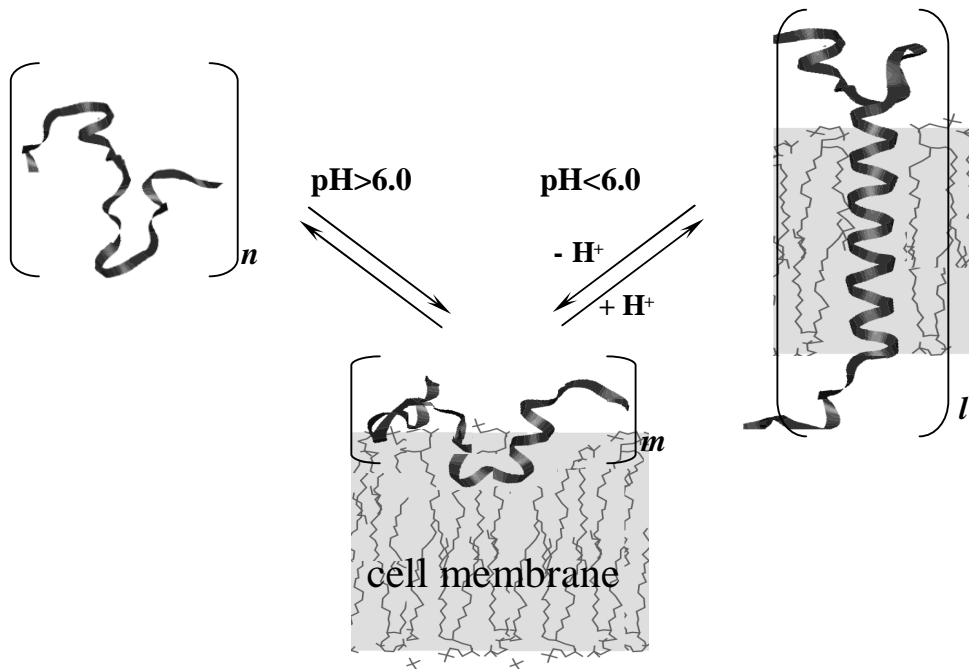
Molecular Nanosyringe for pH-Triggered Injection of Molecules into Cells

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BRC peptide

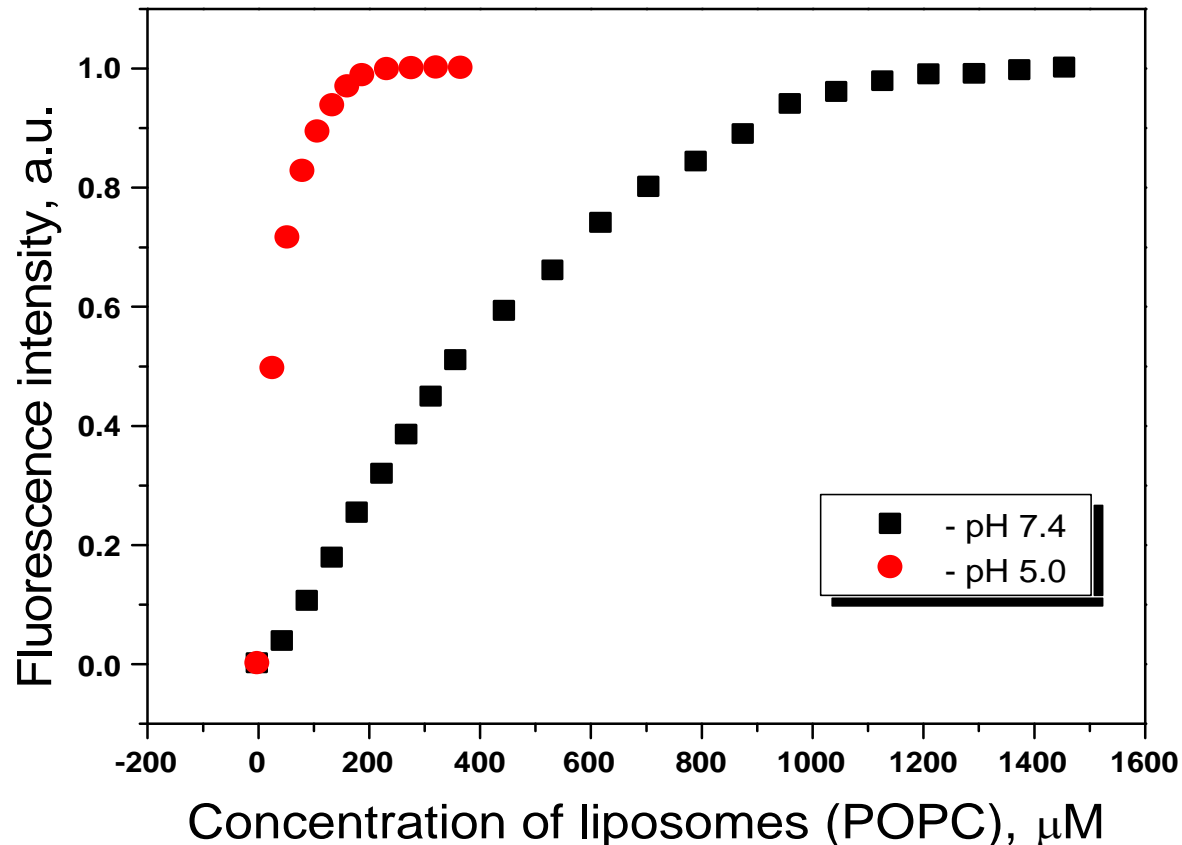


Properties of BRC peptide:

- water-solubility;
- spontaneous insertion;
- fast insertion (seconds);
- reversible insertion;
- pH-dependent insertion into bilayer in a form of transbilayer alpha-helix
- Trp fluorescence is sensitive to binding and insertion

The titration of the BRC with liposomes at pH 7.4 and 5.0

The titration was monitored by changes in intensity of tryptophan fluorescence of the BR-C peptide excited at 295 nm



**Acidic environment is created
in many diseased tissues:**

cancer

heart infarction or stroke

atherosclerosis

inflammation

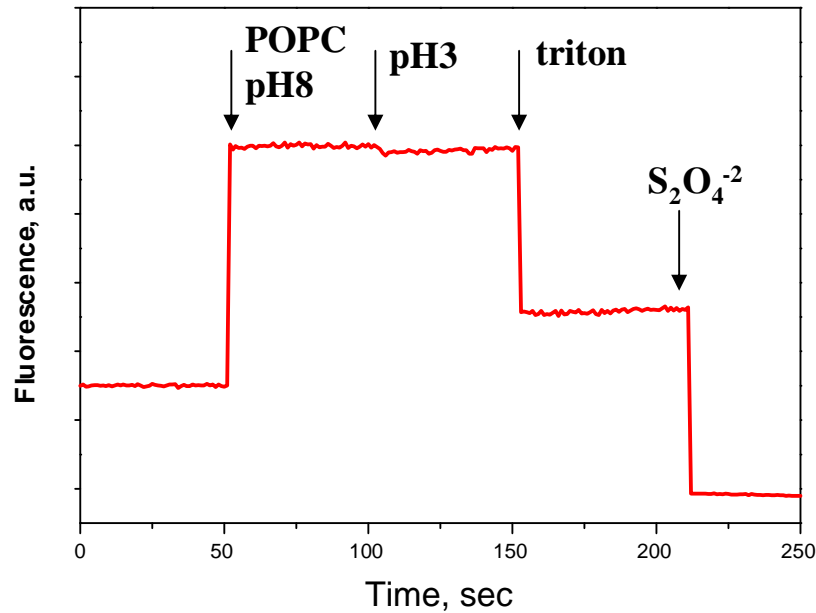
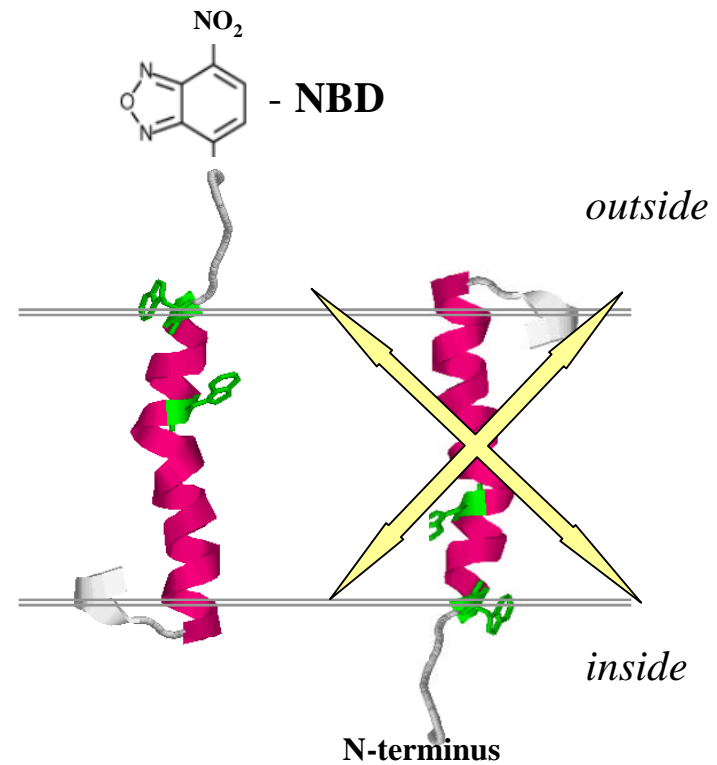
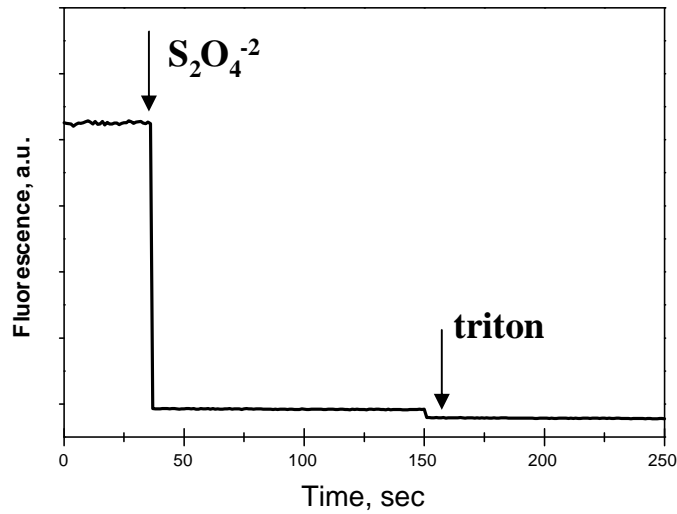
**What is the energy of binding
and insertion of the BRC
peptide into lipid bilayer?**

$$\Delta G_{\text{binding}} \sim -4.5 \text{ kcal/mol}$$

$$\Delta G_{\text{insertion}} \sim -3 \text{ kcal/mol}$$

**What is the topology of the
BRC peptide insertion?**

Topology of insertion



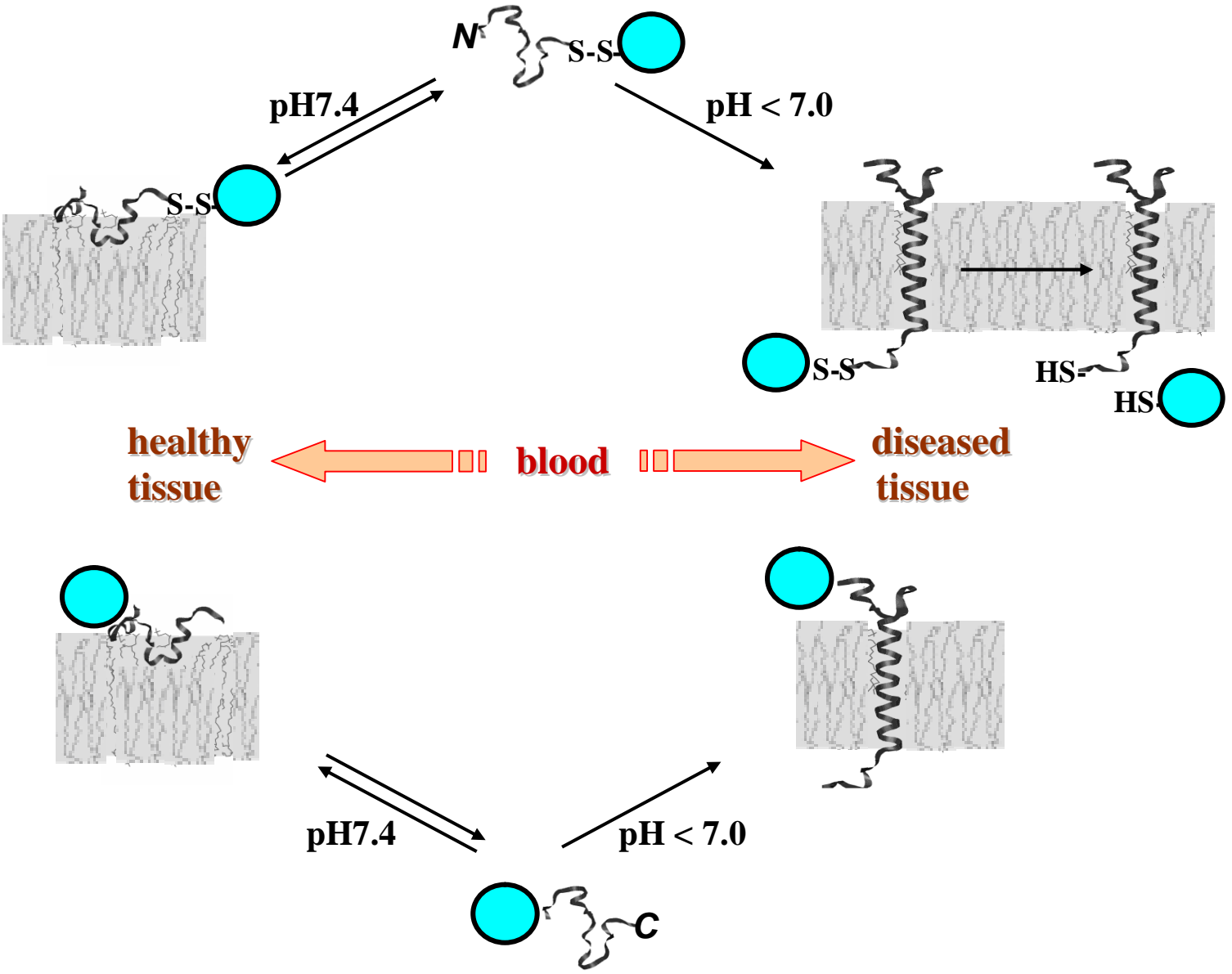
The topology of insertion was evaluated by using the fact that membrane-impermeable dithionite ion ($S_2O_4^{2-}$) can chemically modify the NBD fluorophore and quench its fluorescence

**N-terminus of the BRC
peptide inserted into
liposomes is outside**

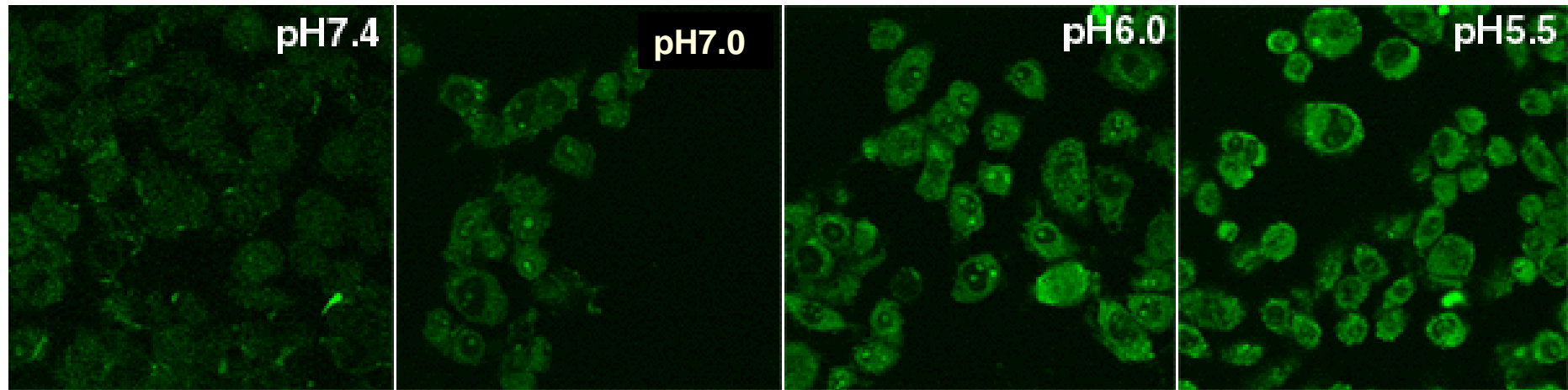
What is the oligomeric state of the BRC peptide in solution and liposomes at neutral and low pHs?

The BRC peptide in 95% is a MONOMER in solution on the surface and inserted into membrane at concentrations <40µg/ml

General concept of Molecular Nanosyringe



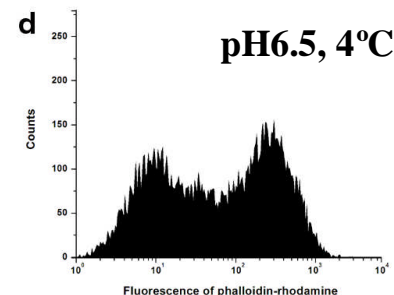
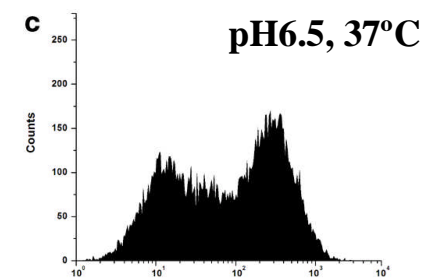
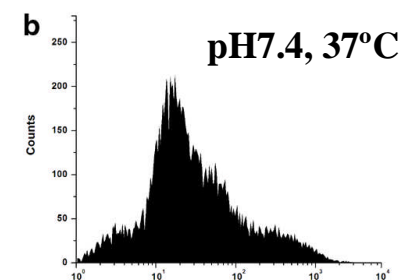
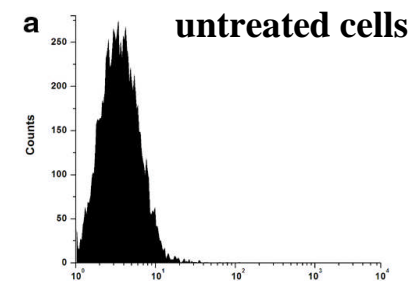
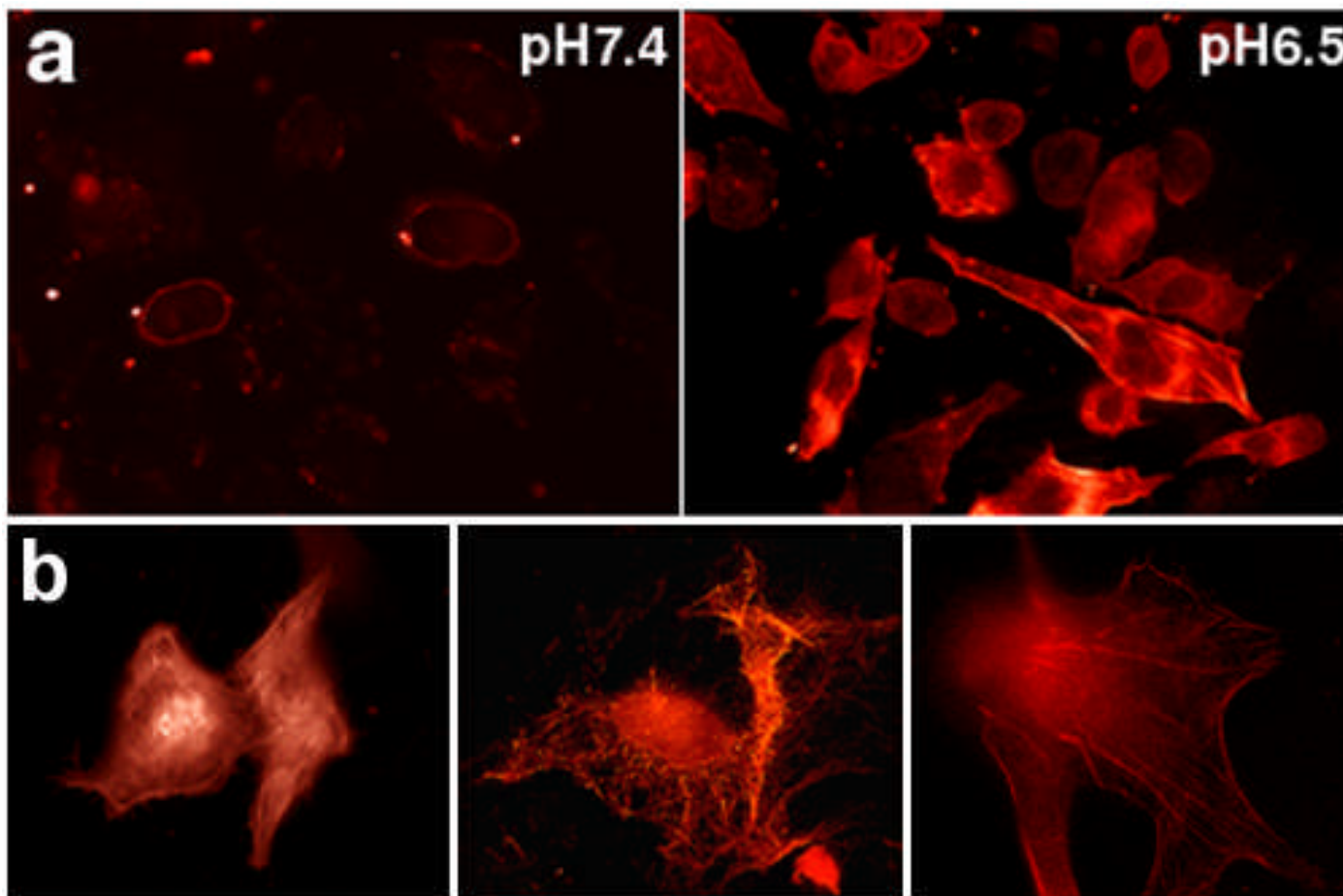
The pH dependent insertion of the BRC peptide and delivery of the dansyl dye into HeLa cells



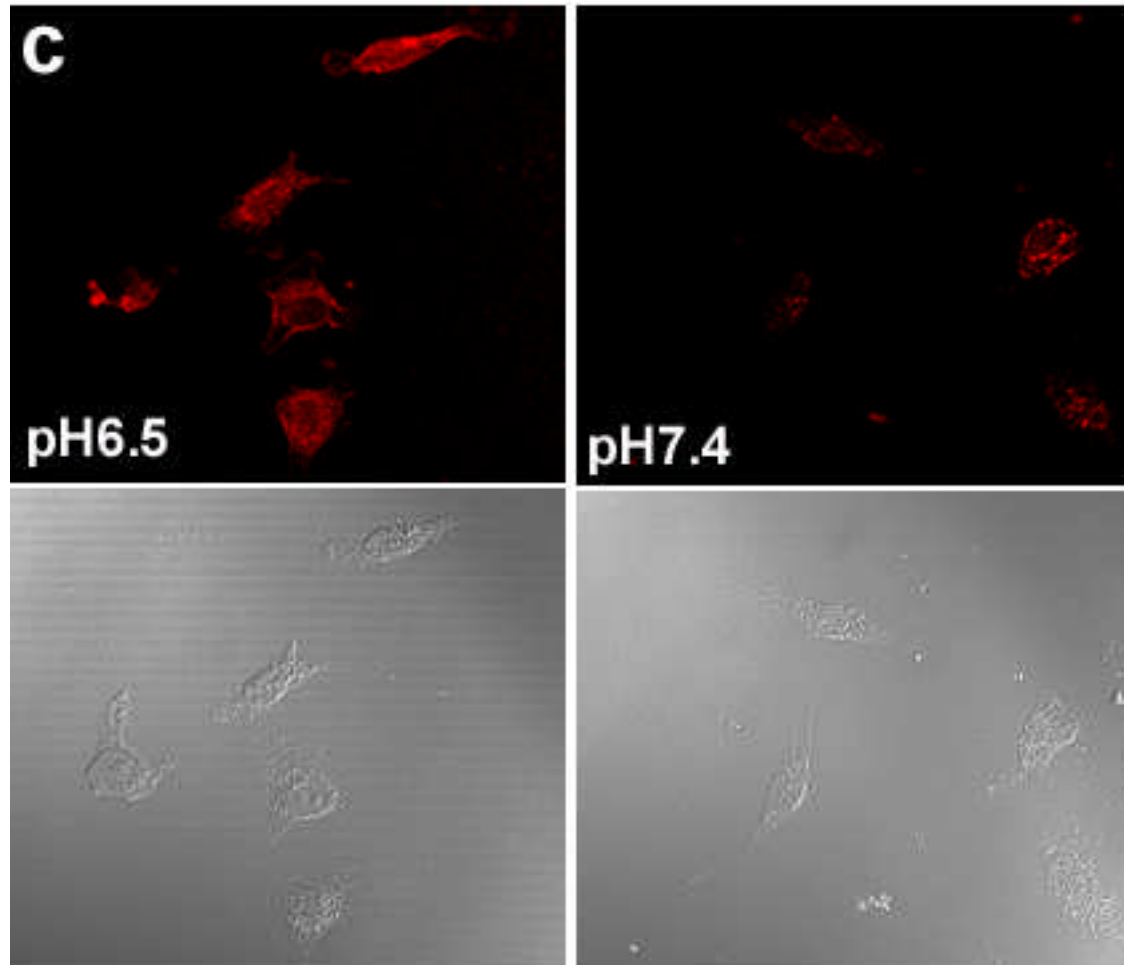
The pictures were taken on the two-photon confocal microscope BioRad MRC-1024 with excitation at 740 nm.

pH-Selective translocation of the Phalloidin-TRITC by the BRC peptide into human (HeLa) and mouse prostate (TRAMP-C1) and breast (JC) cancer cells

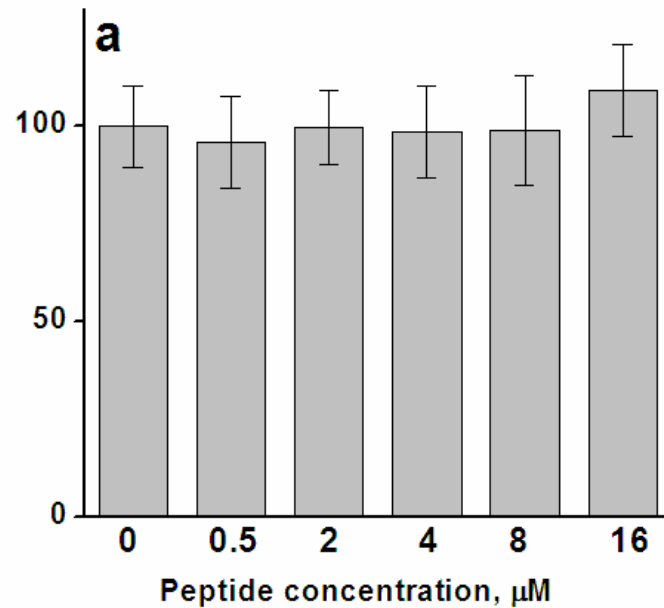
FACS analysis



The ability to wash out non-cleavable BRC-phalloidin-TRITC construct with buffer at pH 7.4 ruled out the endocytotic pathway of molecules translocation

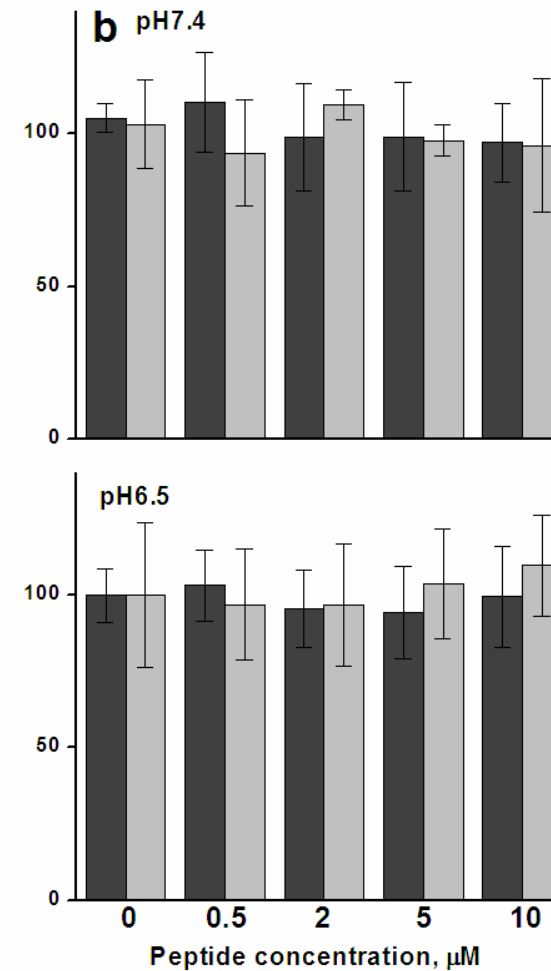


Cell toxicity assay

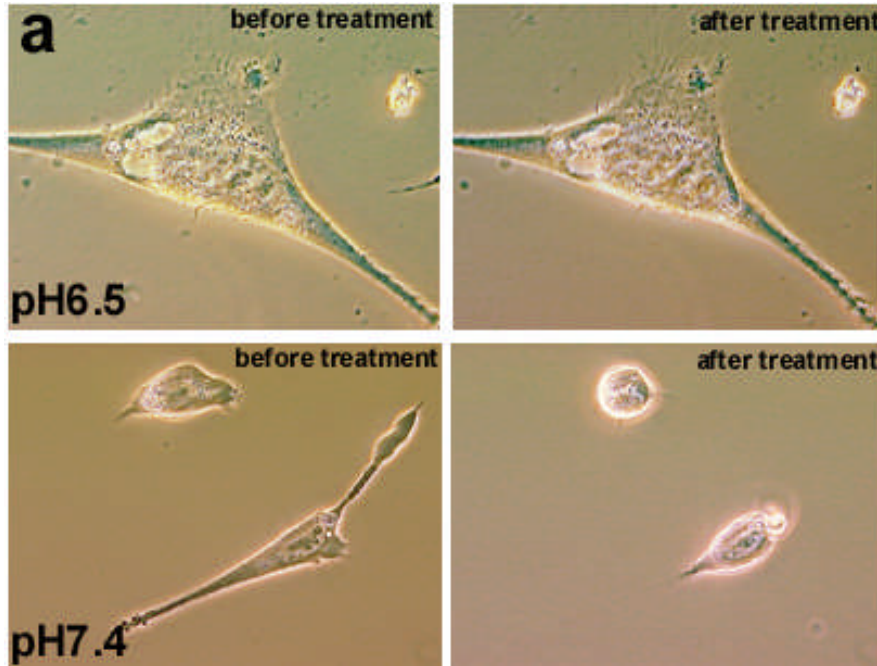


Cell toxicity and membrane leakage tests ruled out the possibility of formations of pores in membrane

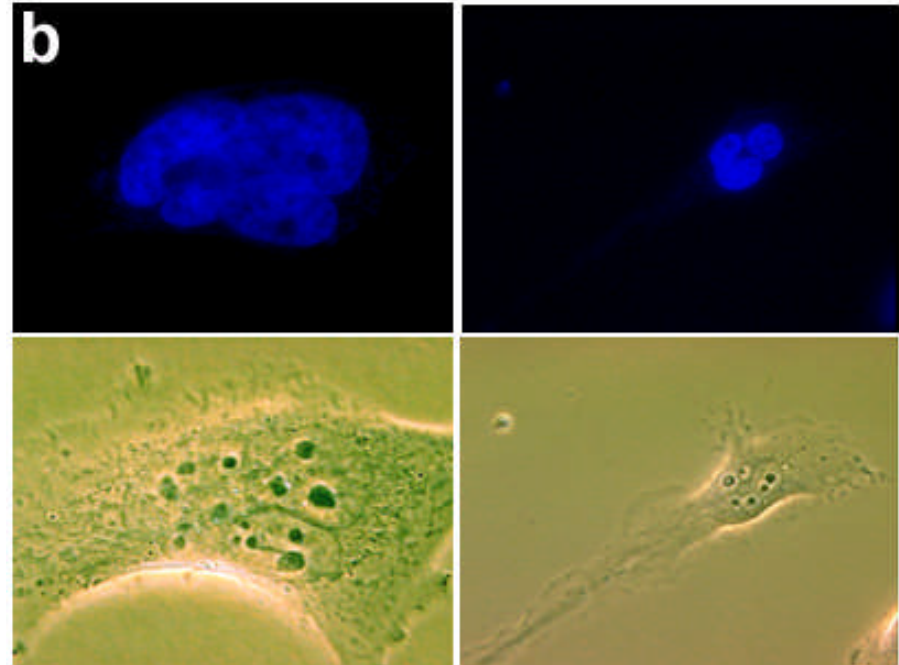
Membrane leakage assay



Biological effects of the translocated into cells phalloidin



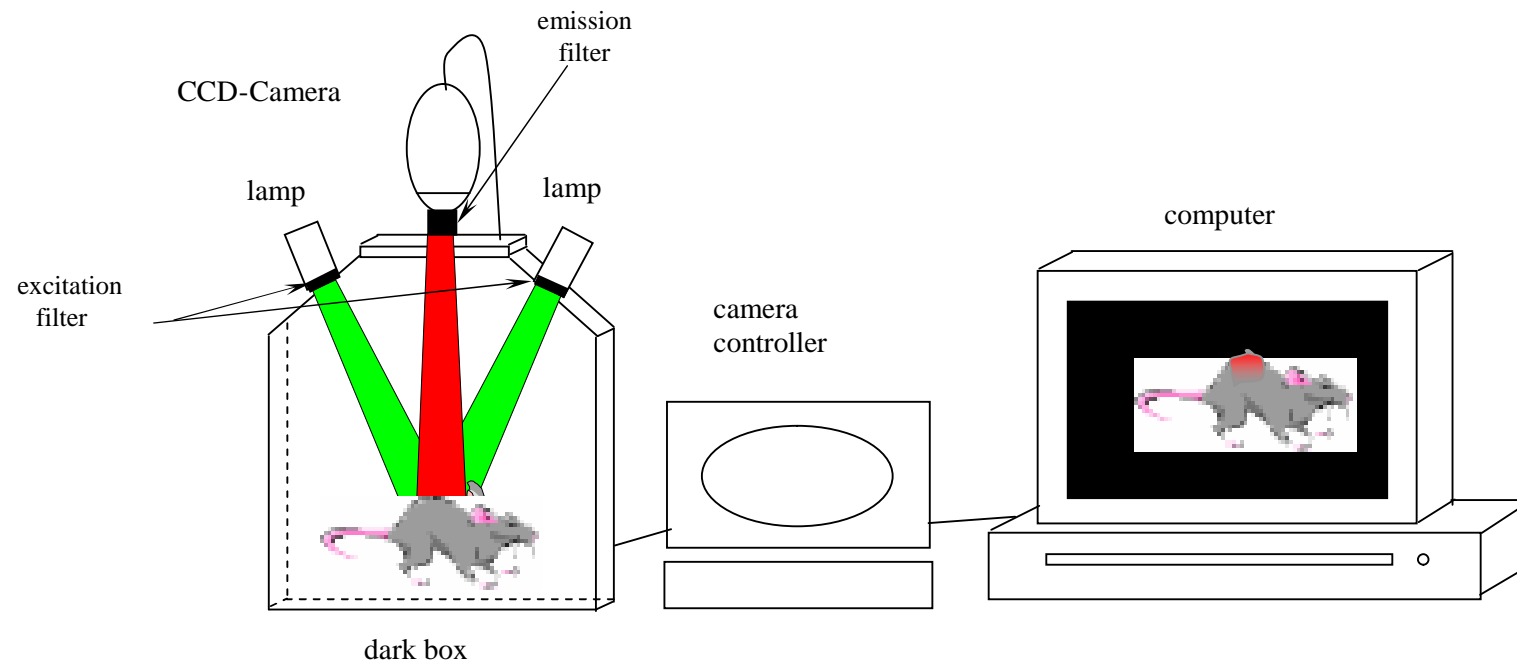
The translocation of phalloidin inside the cells led to inhibition of cytoskeleton dynamics and loss of ability of cells to contract and change shape in response to treatment by EDTA/trypsin dissociation solution.



Multinucleation was observed after 48 hours after treatment of cells with BRC-S-S-Phalloidin at pH 6.5 for 1 hour followed by a change of the medium to DMEM

Whole-body Fluorescence Imaging

To study of the distribution of the BRC peptide in mice and its accumulation in tumor by whole-body fluorescence imaging



Conclusion

BRC peptide is a first example of novel class of transmembrane peptides for pH-selective delivery and translocation of molecules into cells

Acknowledgement



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