Design and Synthesis of Peptide-Based Nanostructures for Sensing and Therapeutic Applications

A bottom-up approach to molecular nanoscale components

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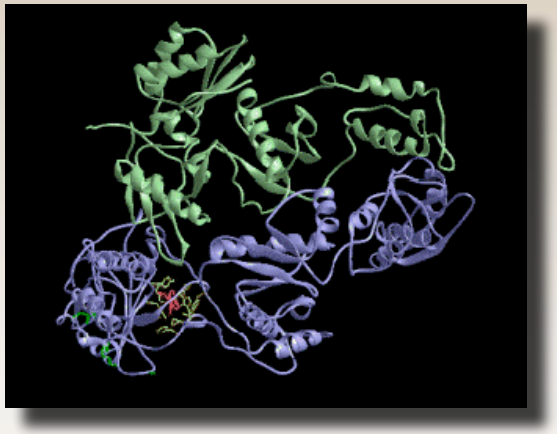
UNIVERSITÉ LAVAL

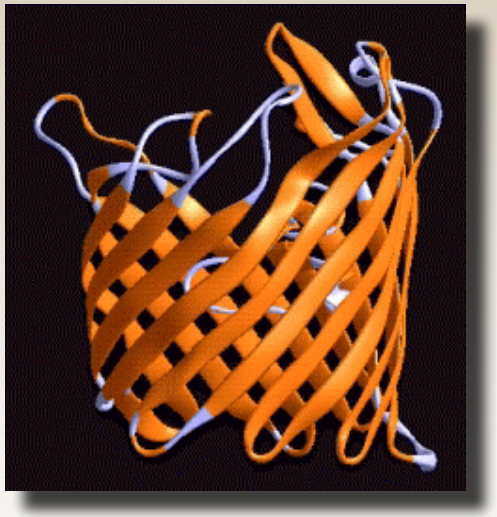
CREFSIP

Polypeptides

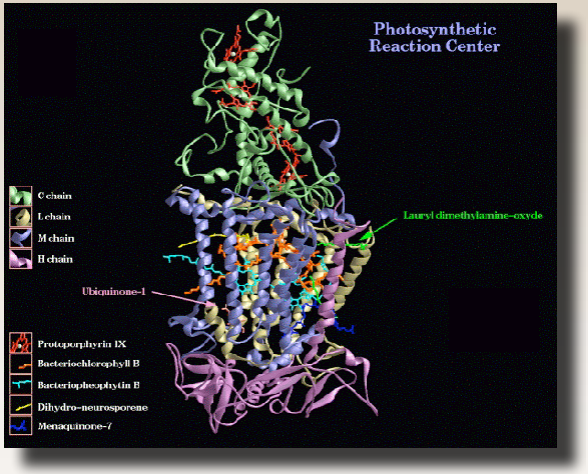
Nature's choice for the construction of functional nanoscale devices



Carbonic Anhydrase



Porin

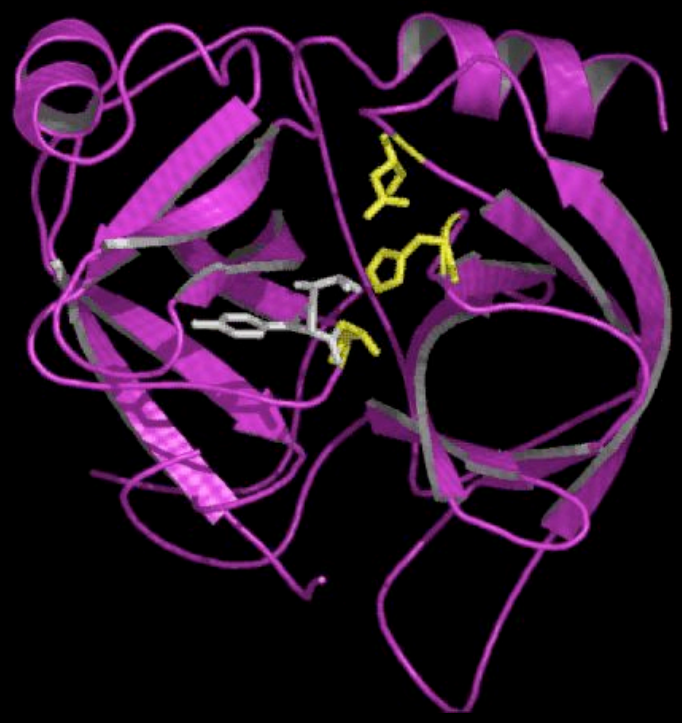


Photosynthetic Center

- Stability
- Wide variety of functional groups

Polypeptide scaffold orients functional groups in the required 3D relationship...

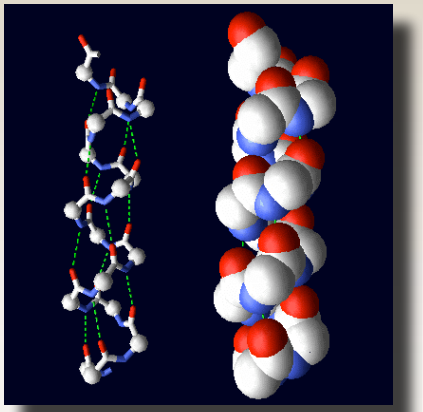
Different Structures: Same Function!

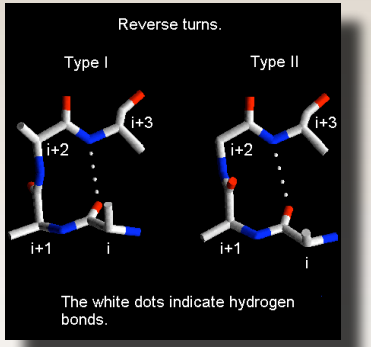


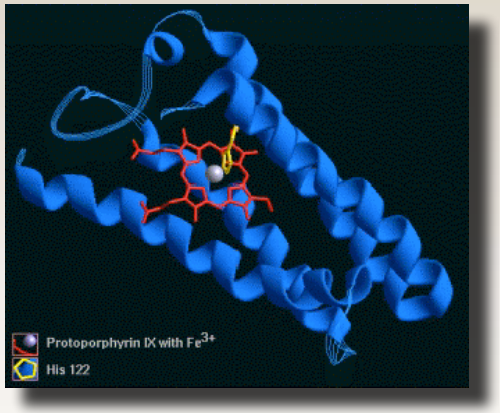
Chymotrypsine

Subtilisine

Modular Construction





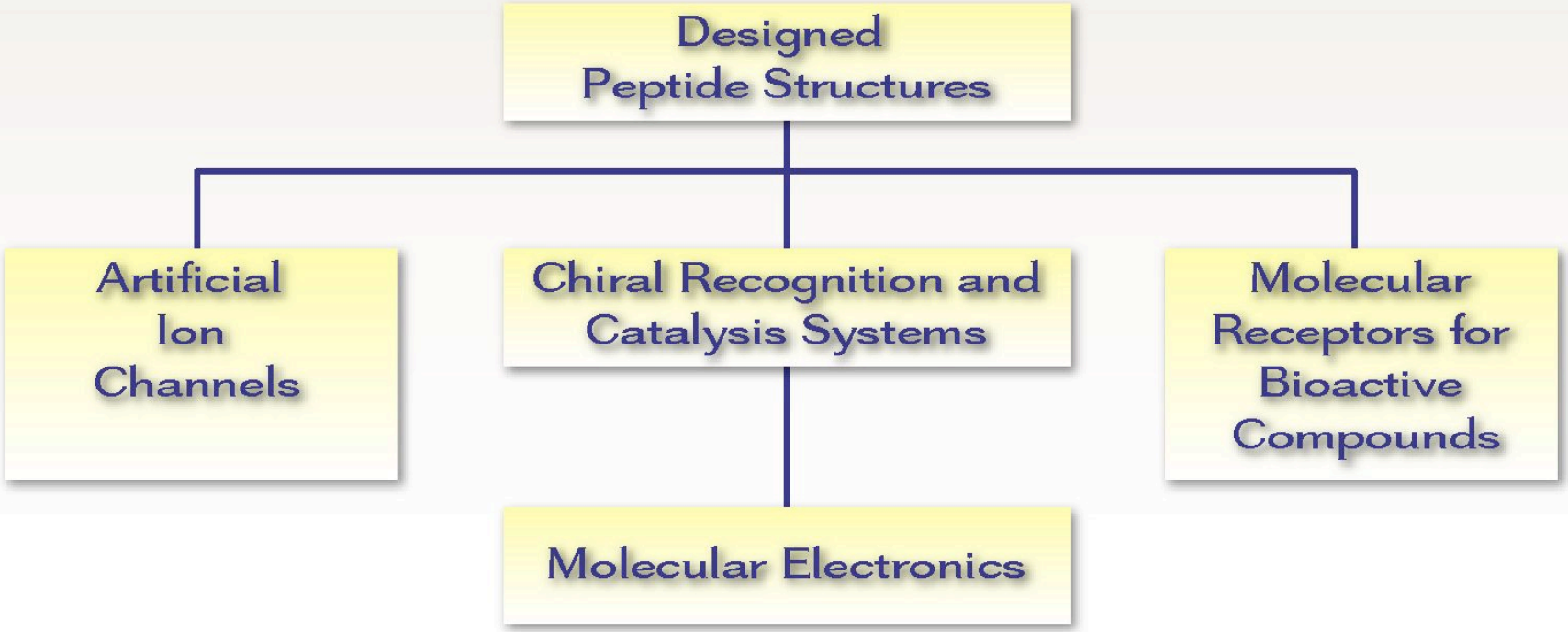


Huge number of possible combinations to create unique nanostructures

Advantages of Polypeptides

- Choice of numerous natural and unnatural amino acids
- Well developped synthetic, purification, and characterization methods
- Ability to predict the solution 3-D structure from the primary sequence
- Allow molecular engineering

Development of Peptide-Based Molecular Devices



Development of Artificial Ion Channels

- Components in molecular sensors
- Novel therapeutic agents
- Gain fundamental insights on biological ion transport processes

Channel Proteins

- Complex membrane proteins (MW>100,000; multiple sub-units)
- Difficult to isolate the active form
- Responsable for ion transport across cell membranes
- Extremely efficient: $>10^7$ ions/sec!
- Involved in numerous diseases

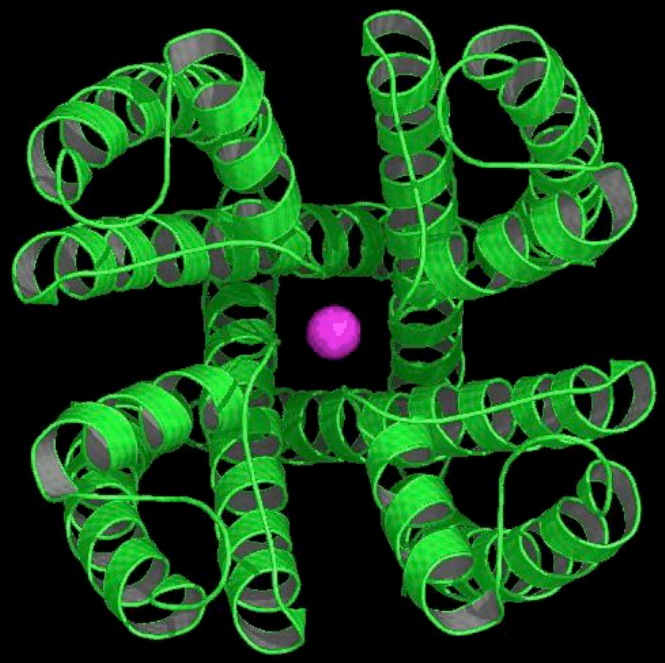
Problems with Natural Channel Proteins

- Large and complex membrane proteins
- Difficult to isolate and sensitive to denaturation
- Available in small (impure) quantities
- Very expensive
- Molecular engineering tedious

Design and Synthesis of a Family of Artificial Ion Channels

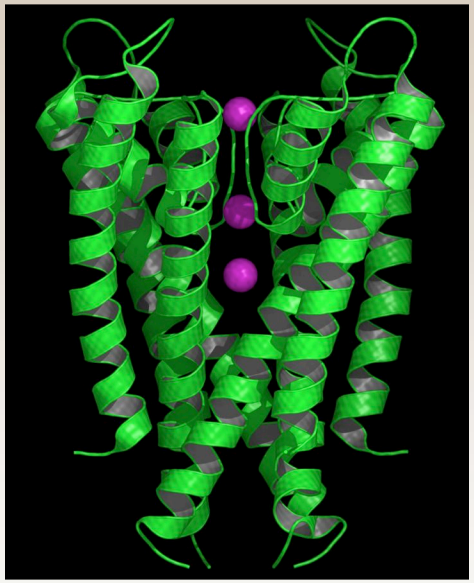
- By a simple synthetic strategy
- With possible "post-synthesis" modifications
- With different properties (ion selectivity)
- With simple monomeric active forms

KcsA: A Channel Protein Model

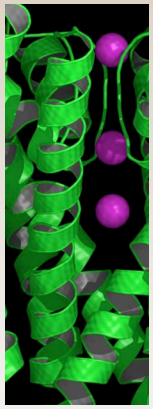


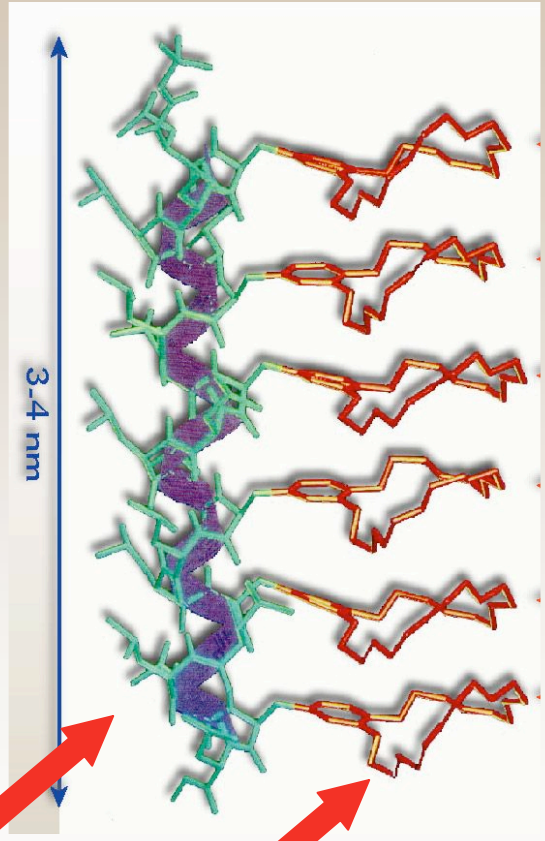
R. Mackinnon et al, Nature, 2001

A Minimalist Approach



KcsA





Helical peptide nanostructure

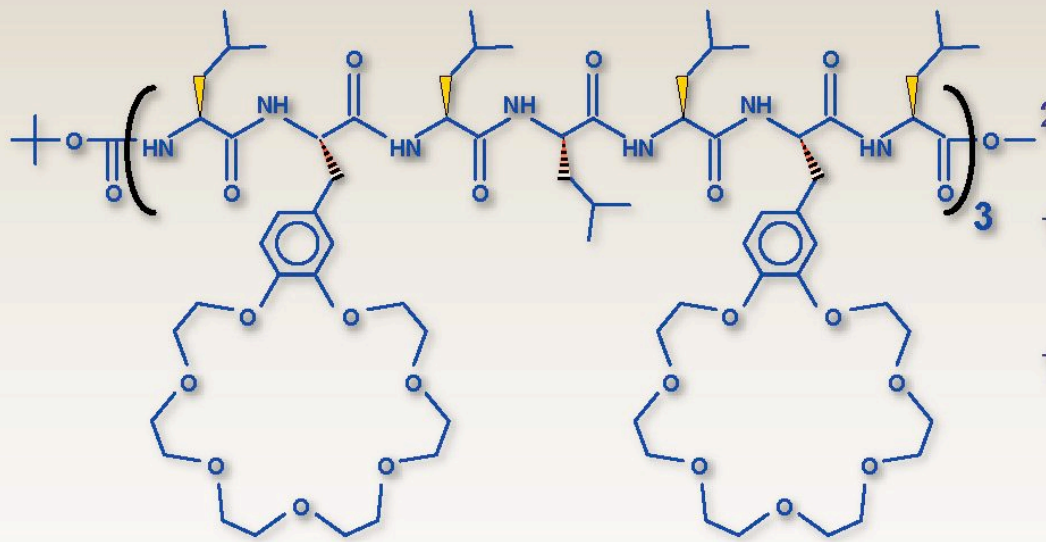
Crown ether array

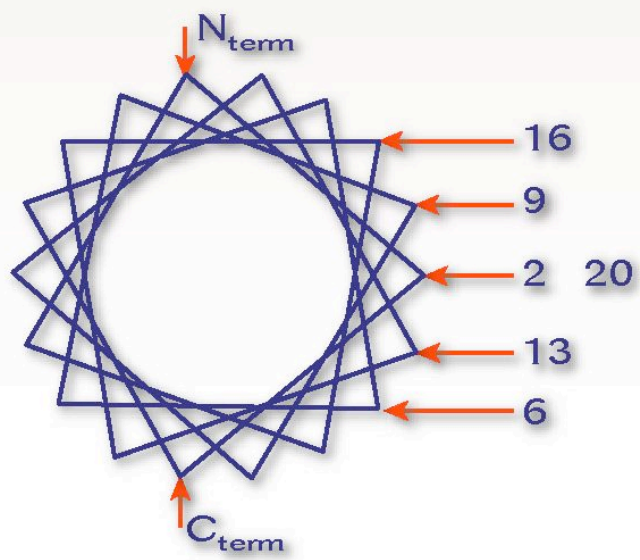
The Prototype

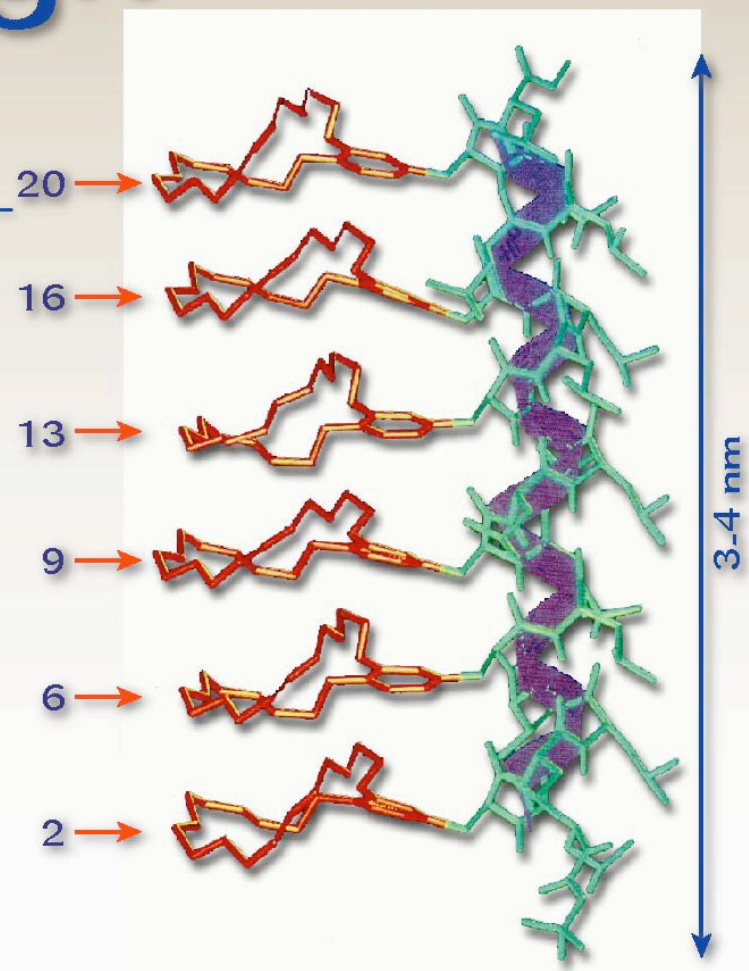
N-BOC-L-CE-L_3-CE-L_2-CE-L_3-CE-L_2-CE-L_3-CE-L-OMe

- 21 Residue peptide
- Composed of L-Leucines and 6 (21-crown-7)-L-Phenylalanine (CE)
- Predicted to adopt an α-helix conformation under which the crown side chains are all aligned

Channel Design

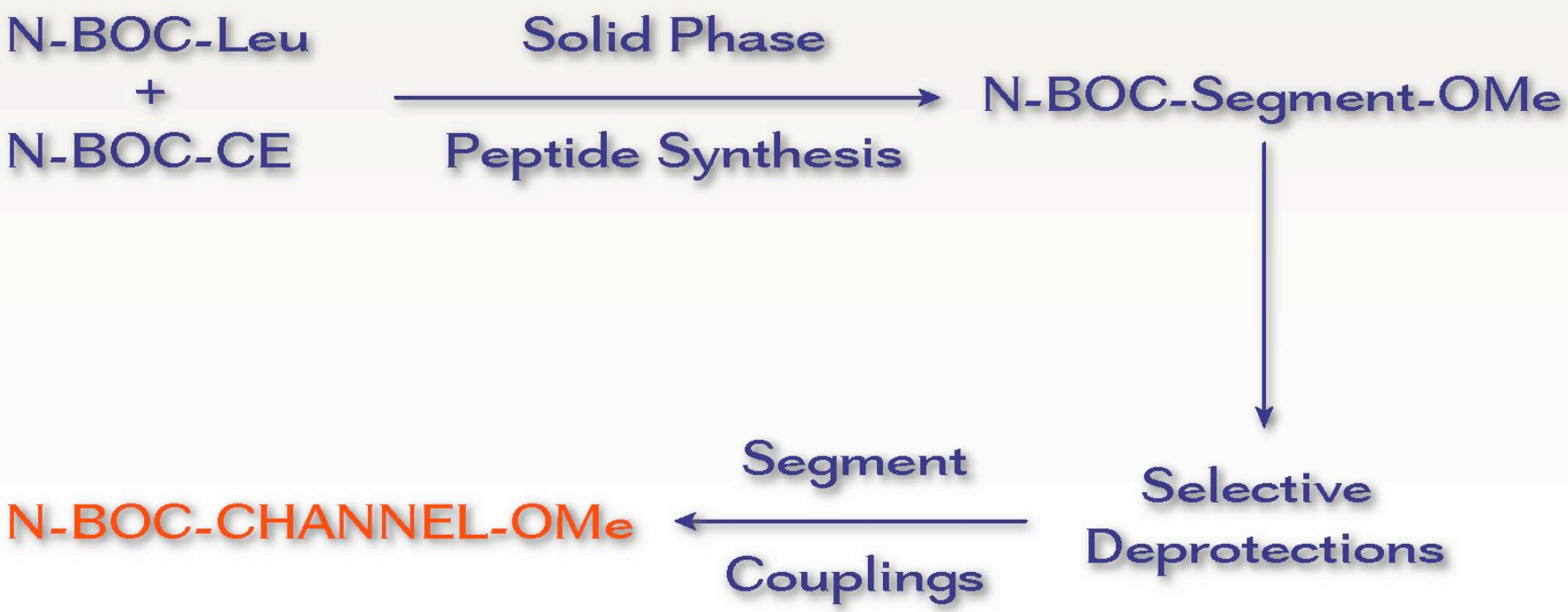




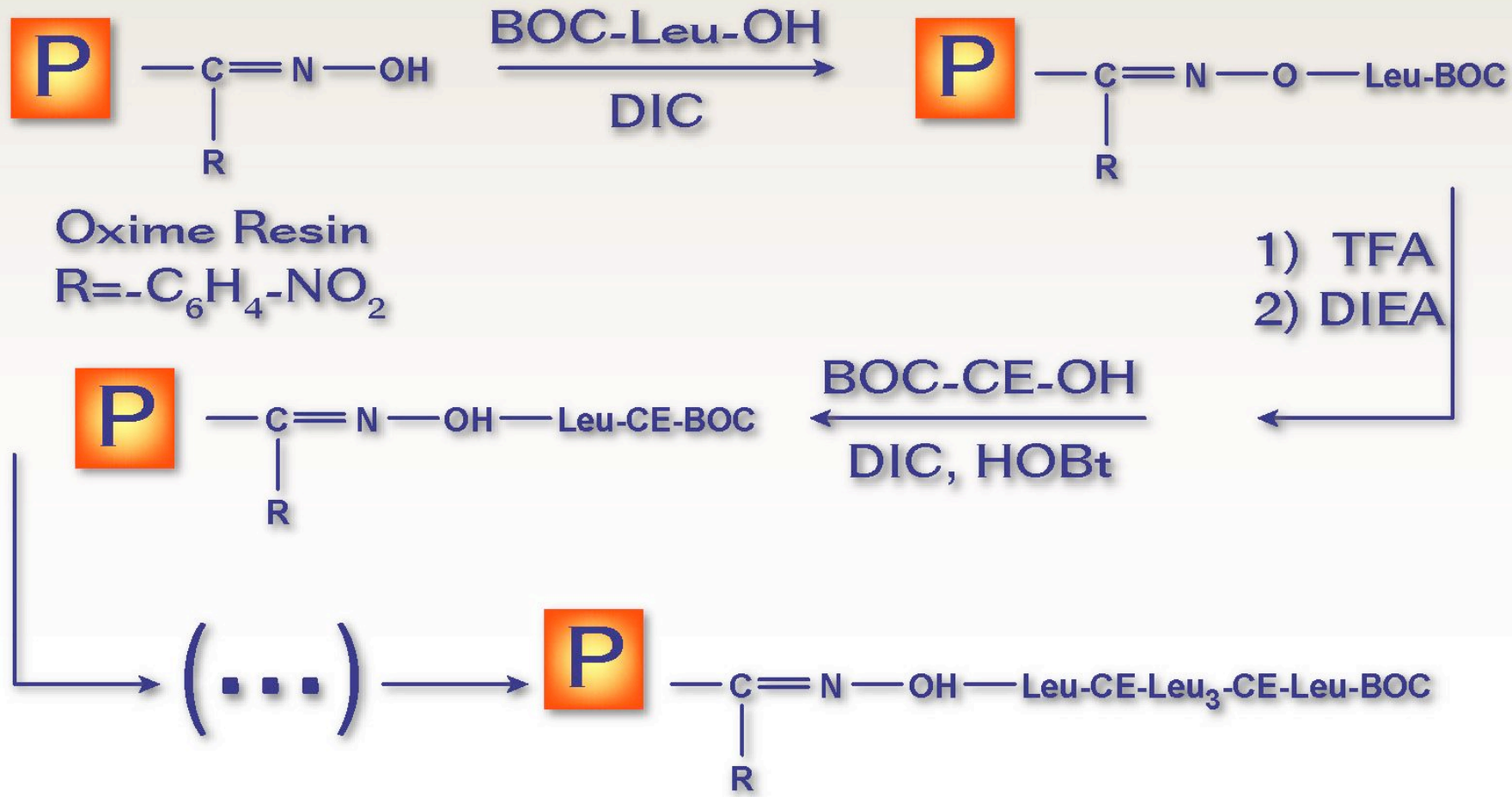


Jean-Christophe Meillon, Normand Voyer, Angew. Int. Ed. Engl., 1997, 36, 967.

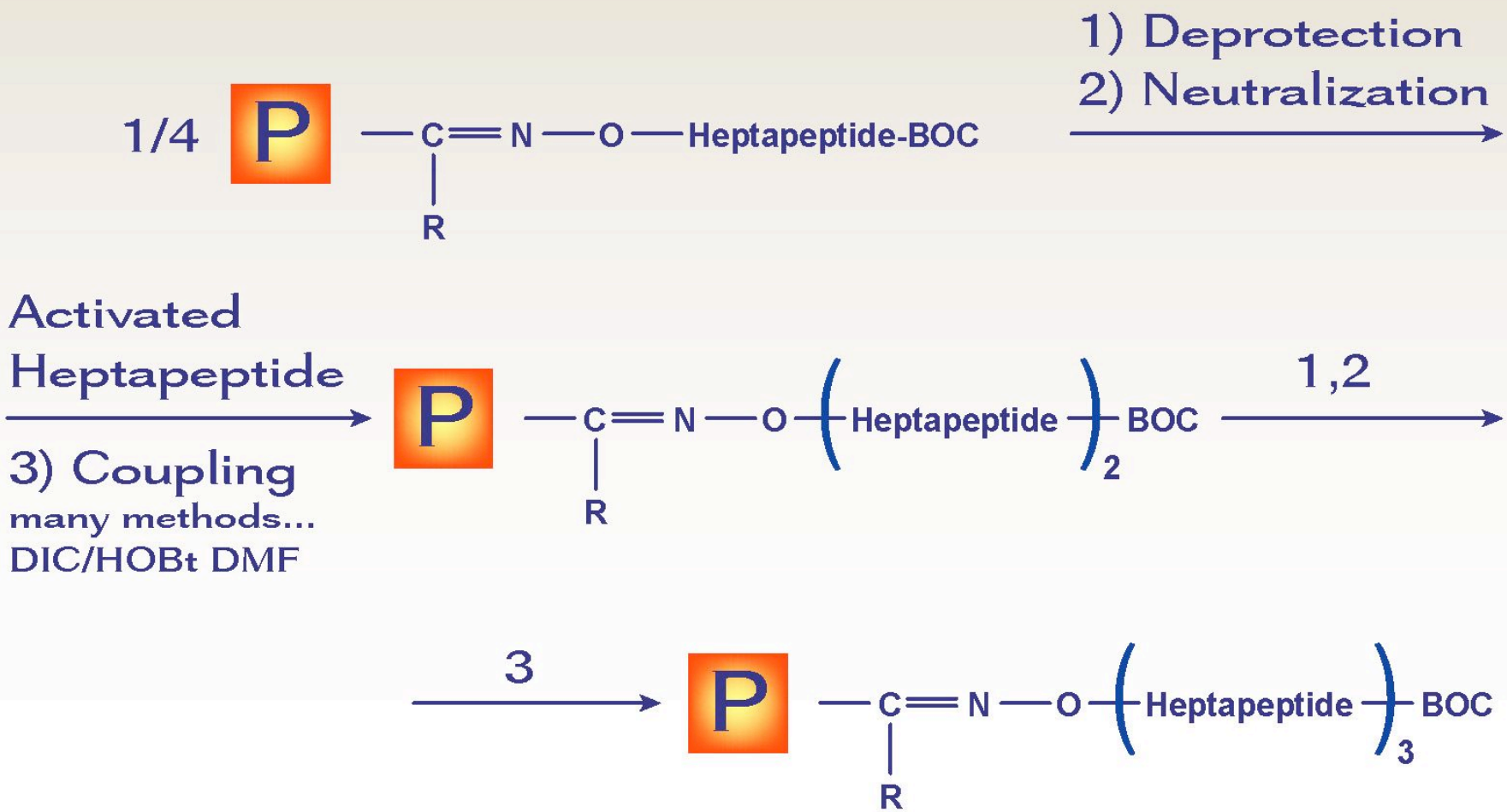
General Synthetic Strategy



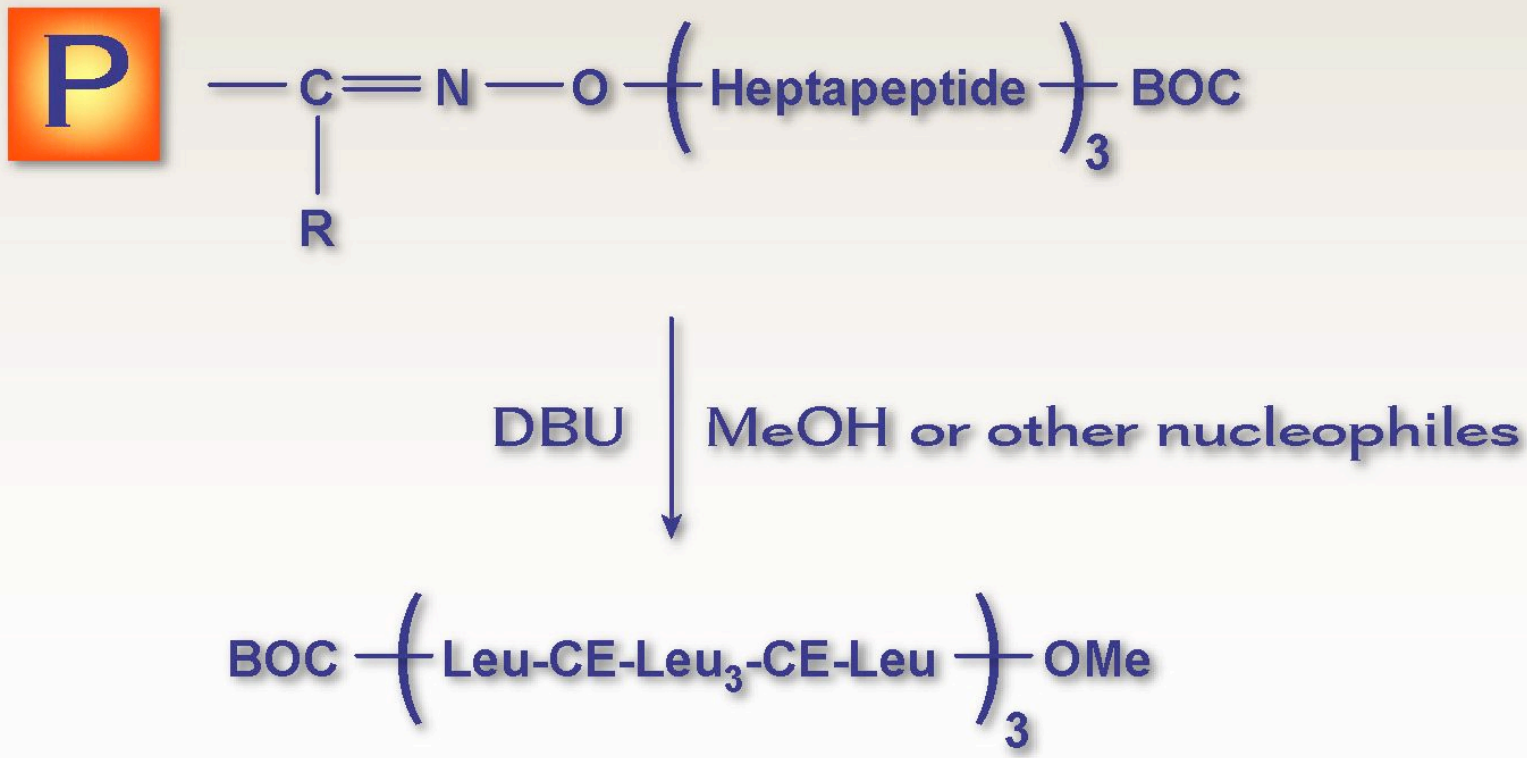
Protected Segment Synthesis



Segment Couplings



Final Cleavage

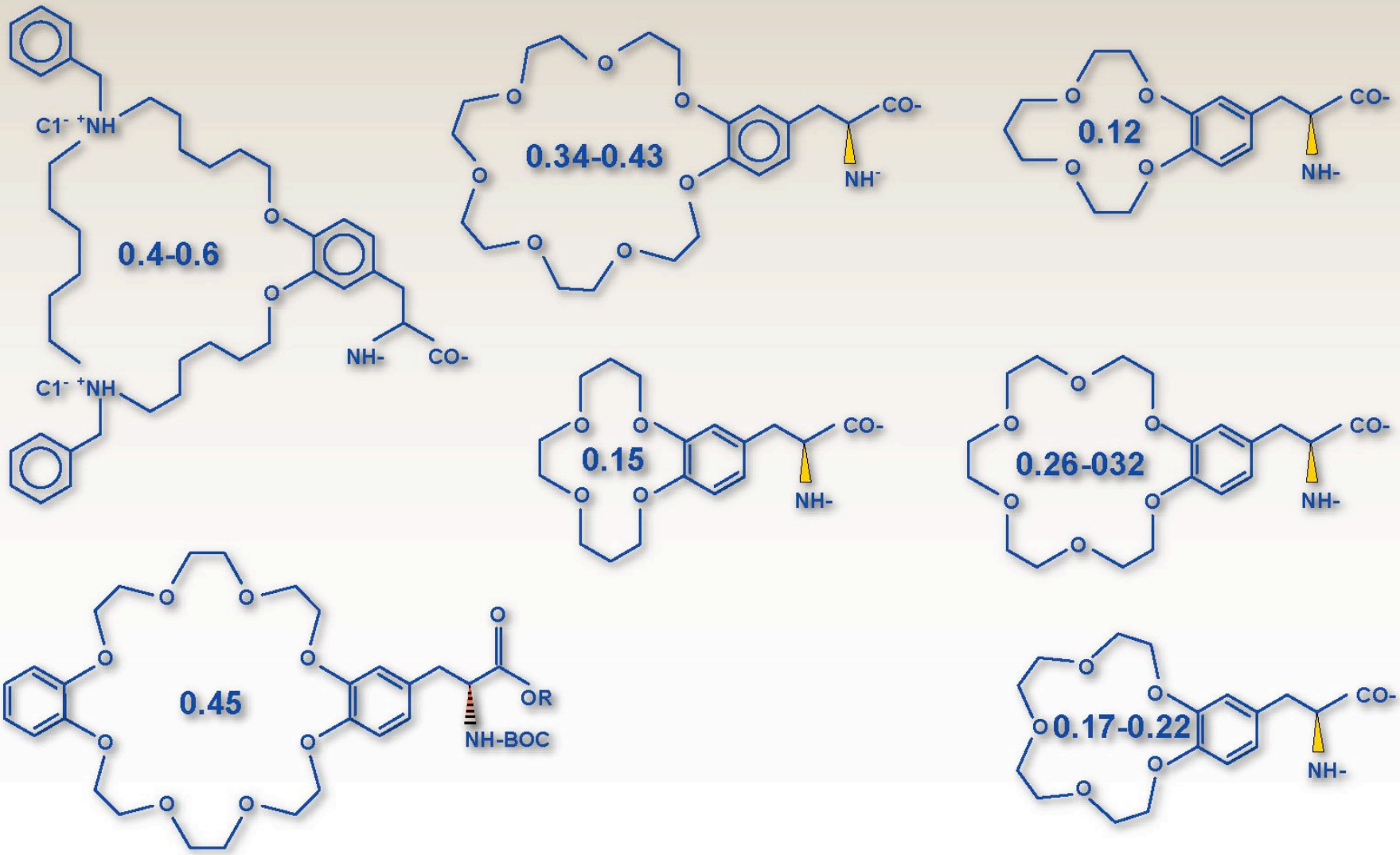


Desired 21-mer fully protected!

Characterization

- ^{1}H ^{13}C NMR Spectroscopy
- FAB, ES, MALDI Mass Spectroscopy
- Molecular Weight Range=3500-5000 g/mol
- 20 Steps
- Overall Yields=>10%
- Numerous derivatives prepared
- Available at gram scale!

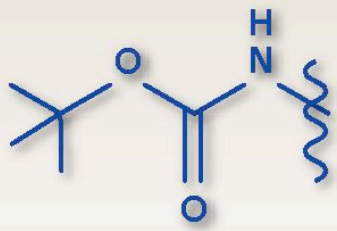
Nanopore Technology

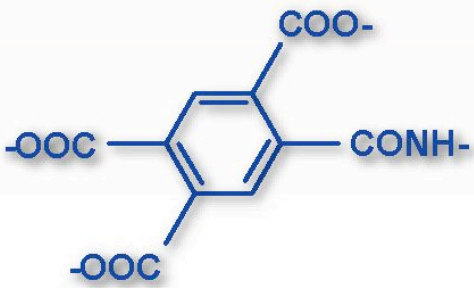


Diameters in nm

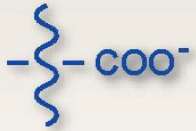
End Groups

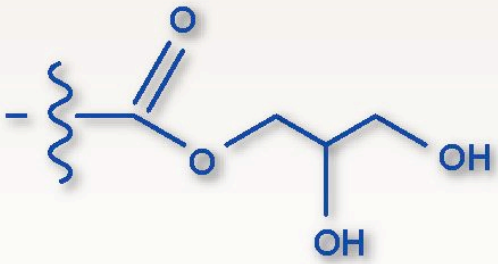
N-terminal groups

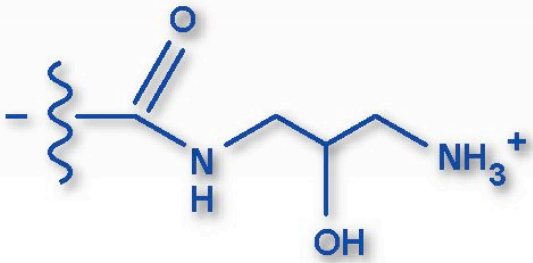




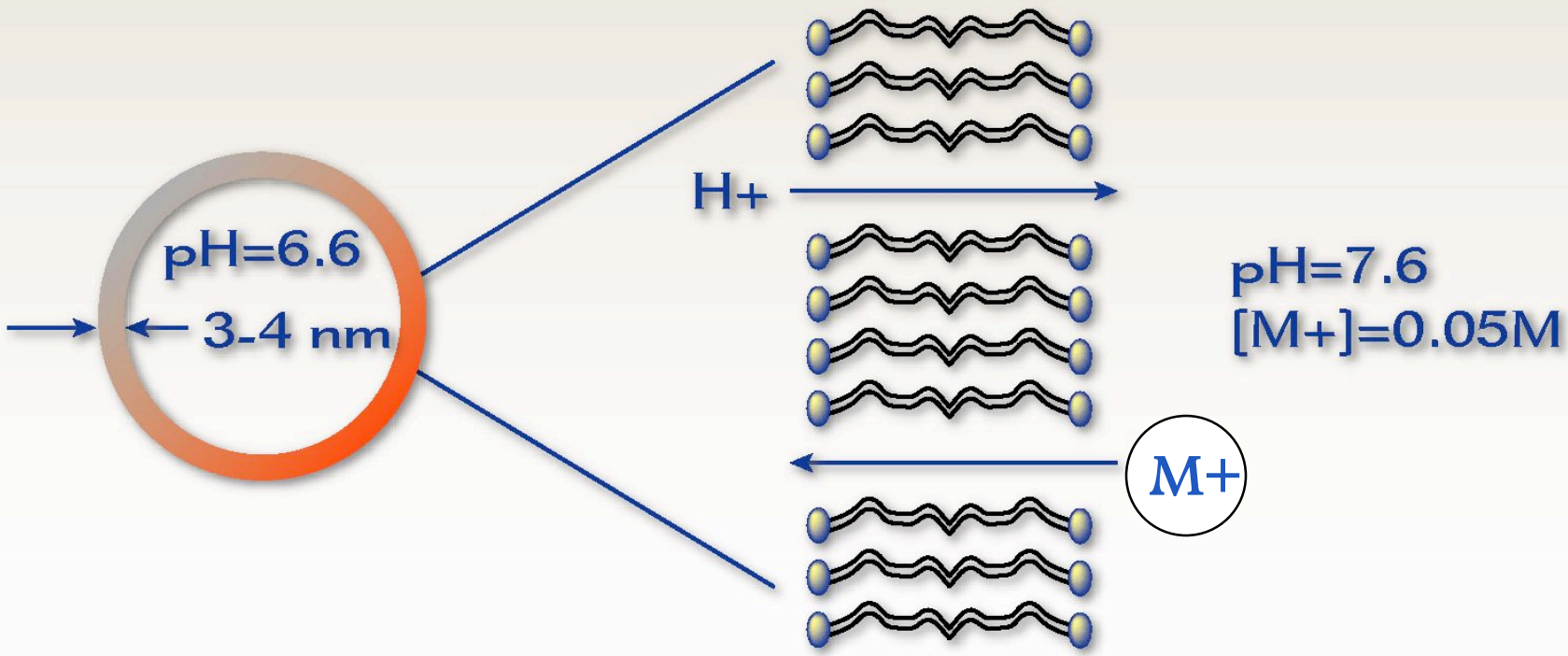
C-terminal groups



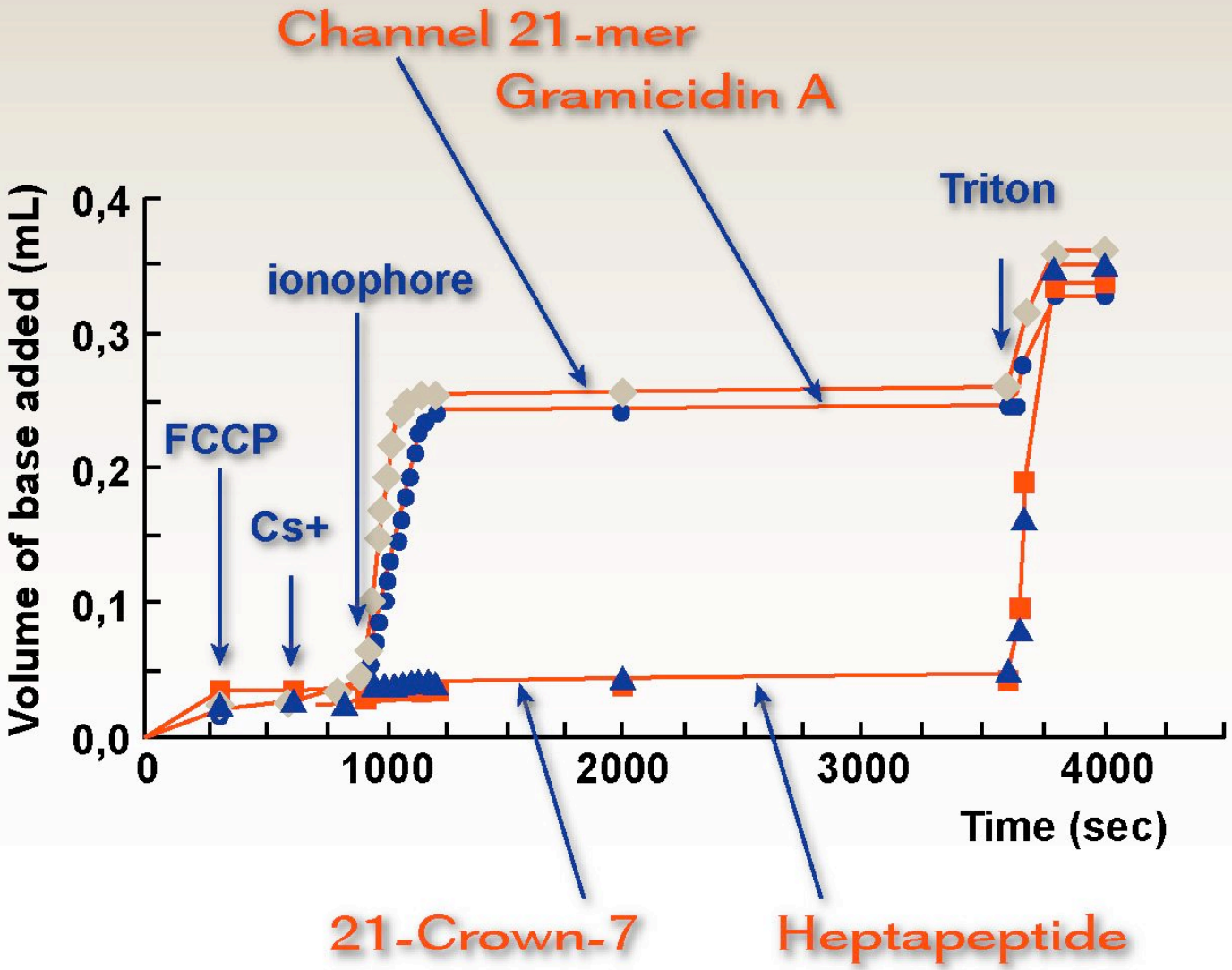




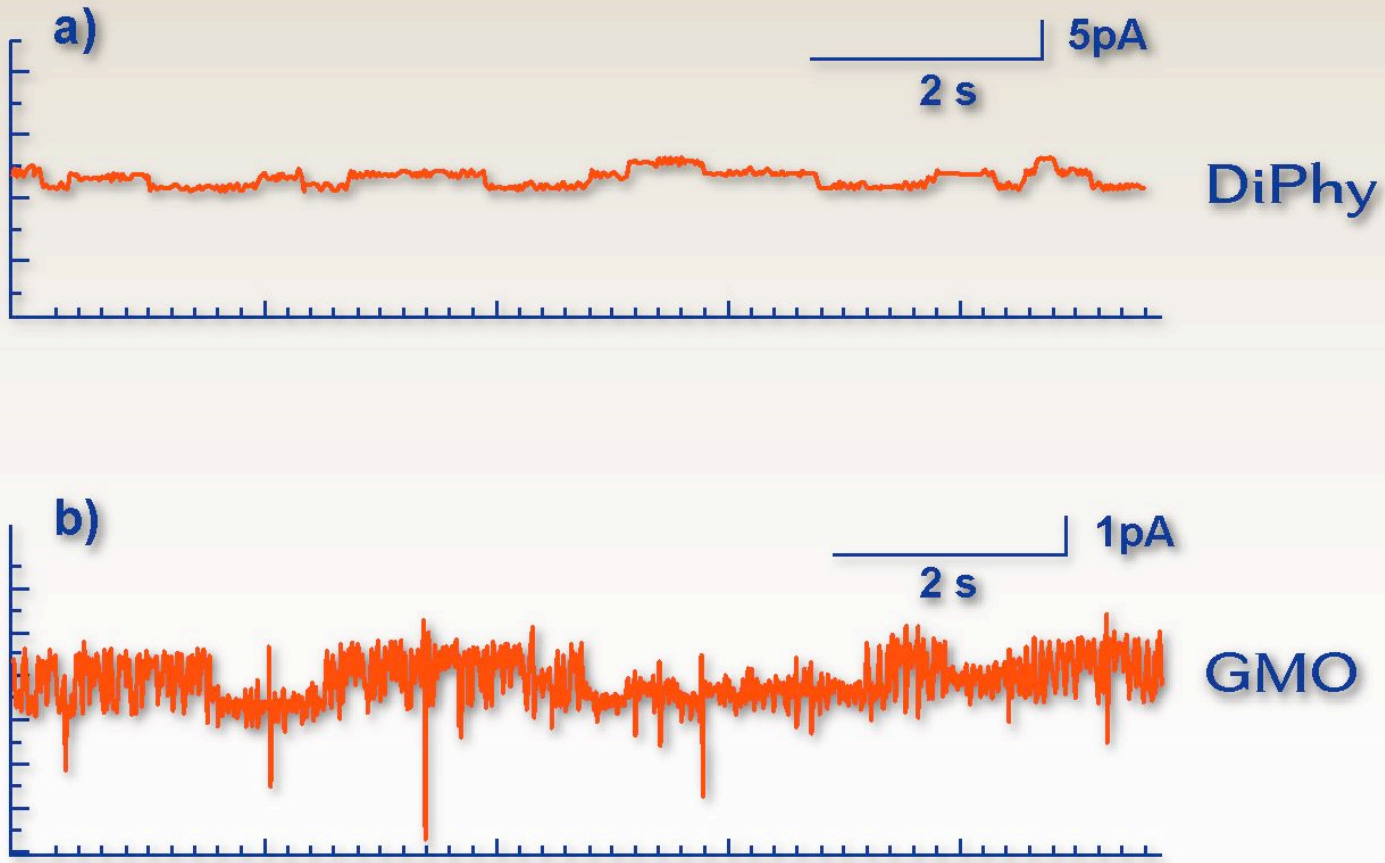
Ion Transport Ability in Vesicles

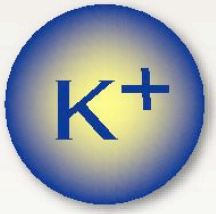


Vesicle Studies



Membrane Conductivity





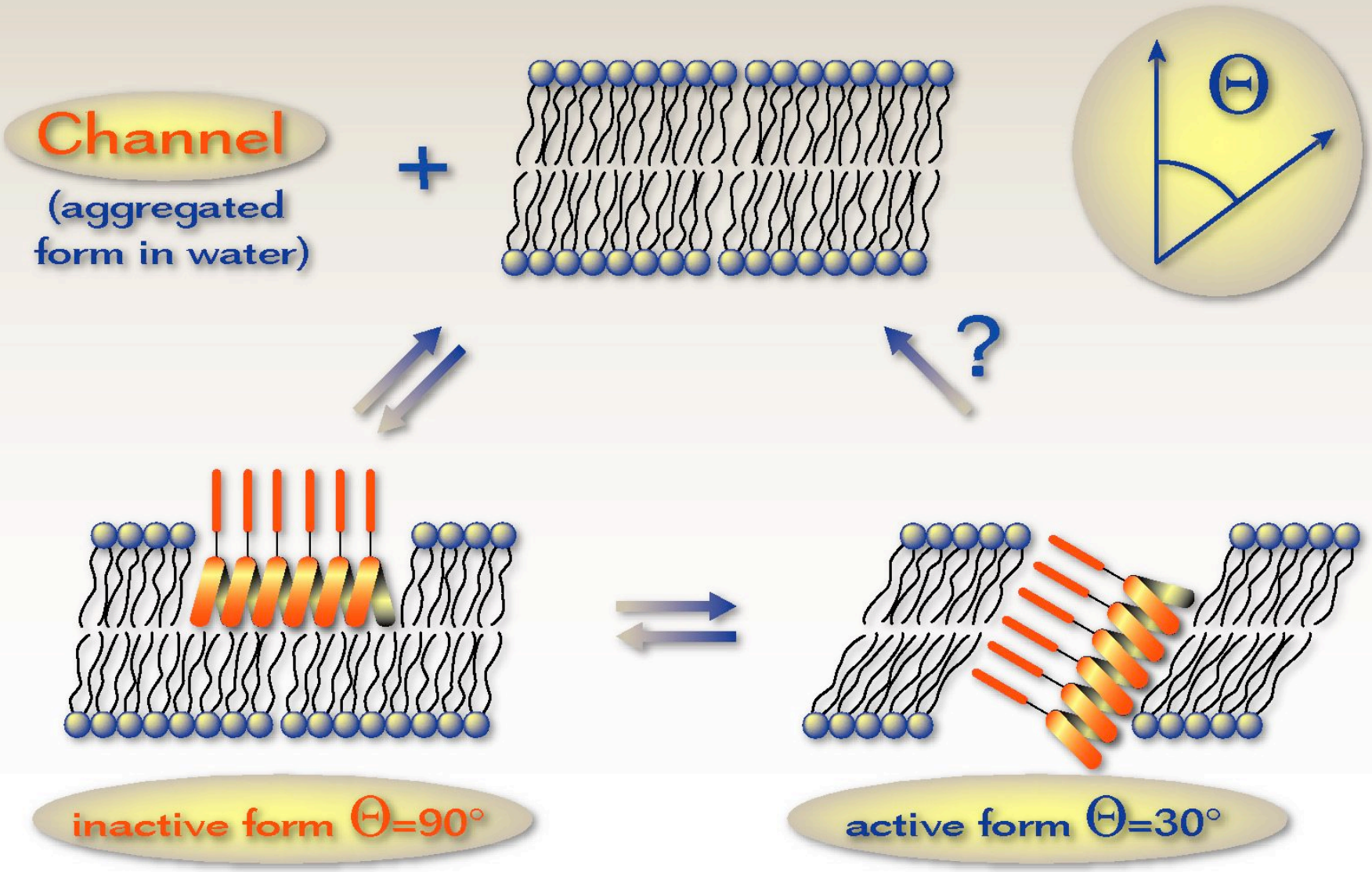
Conductivity: 2.5 pA; average open time: 1 sec (DiPhy)

Biophysical Studies

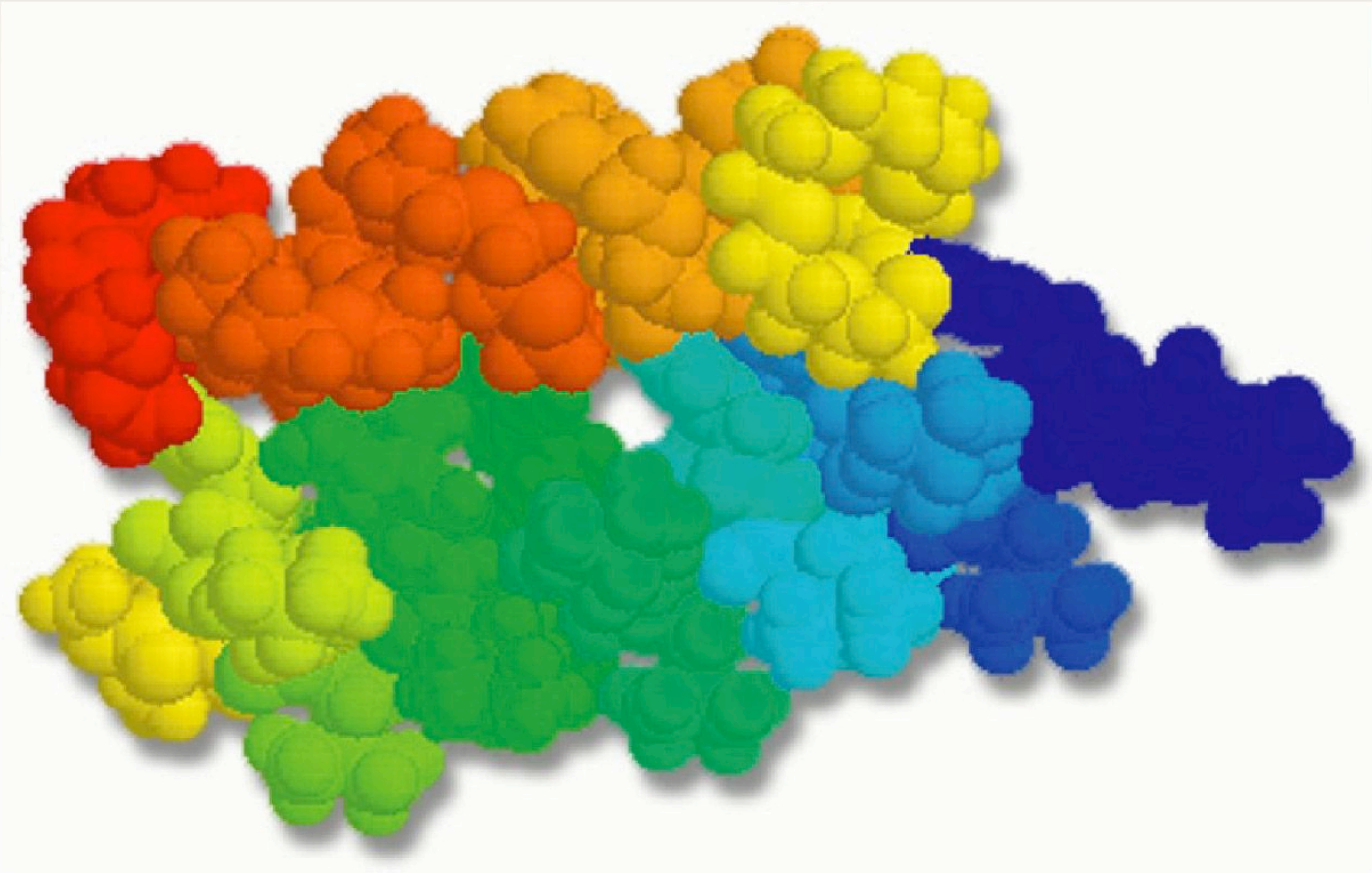
- CD spectropolarimetry (solvents, concetration, vesicles, salts, etc)
- FT-IR (solvents and vesicles)
- Polarized ATR (different peptide/lipid ration, different lipids)

Strongly α-helical, monomeric in hydrophobic solvents

Proposed Mechanism



What can we do with these compounds?



Nanoscale Therapeutics

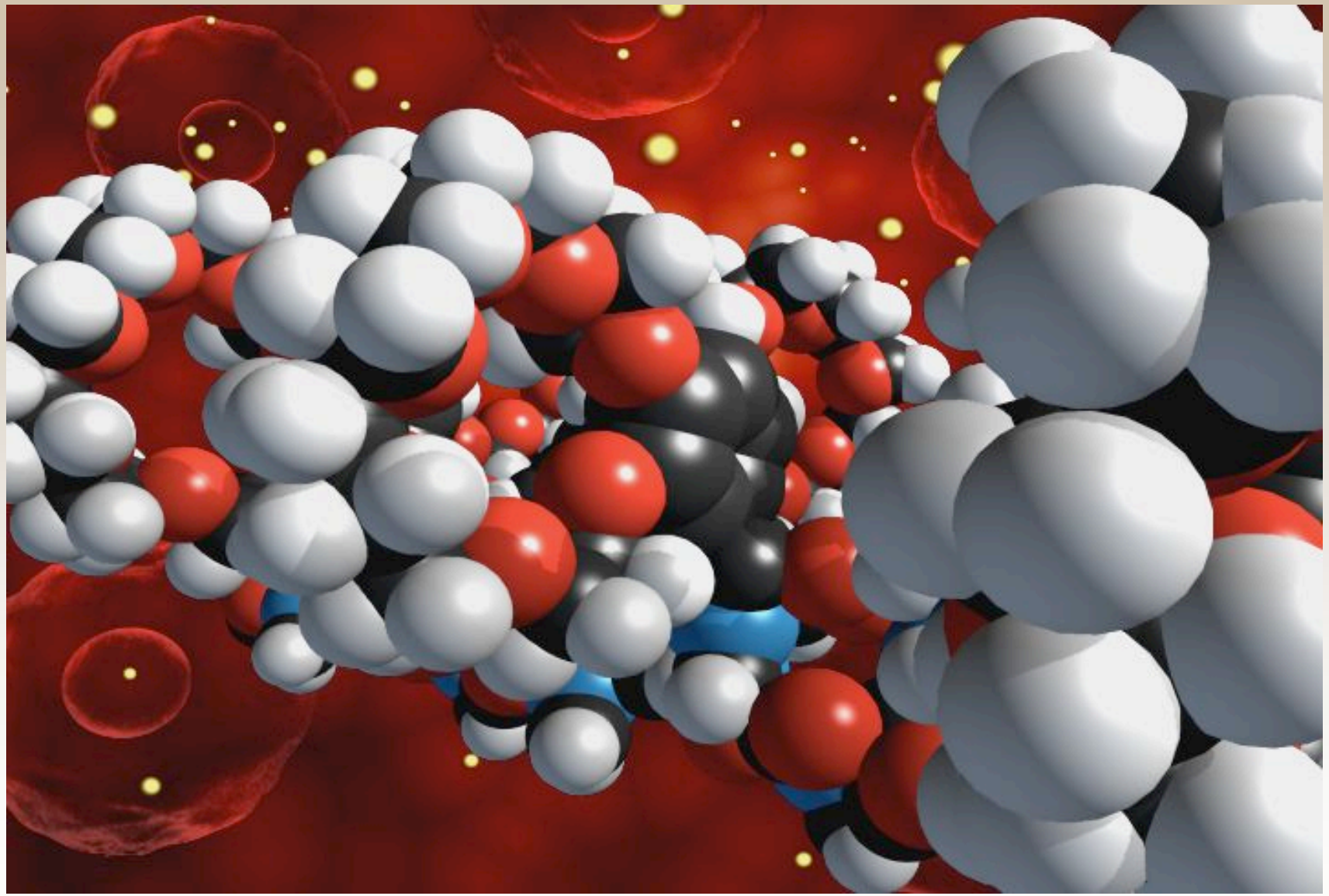
Use synthetic channels as membrane « hole punching tools »

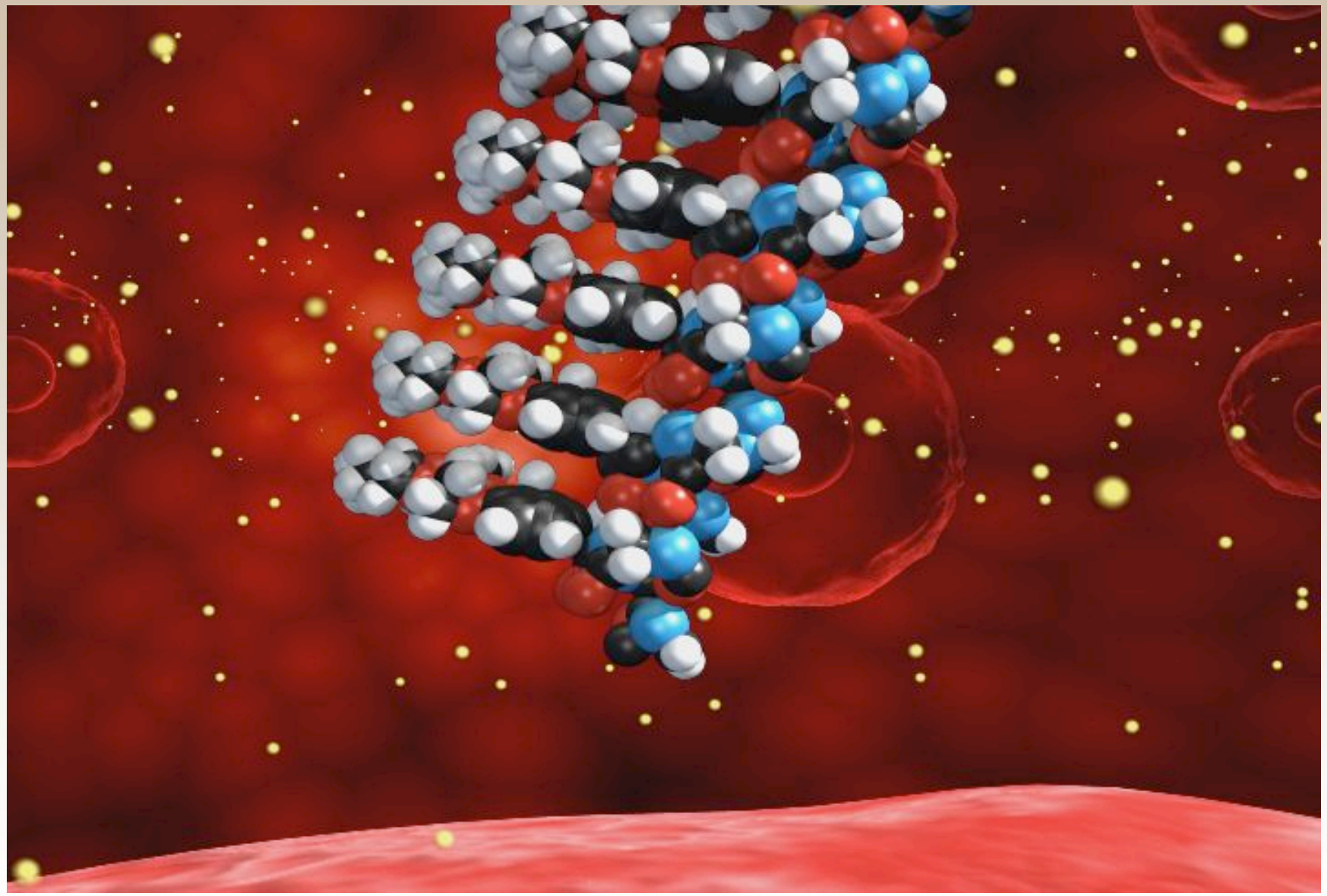
↓

Wide variety of target cells

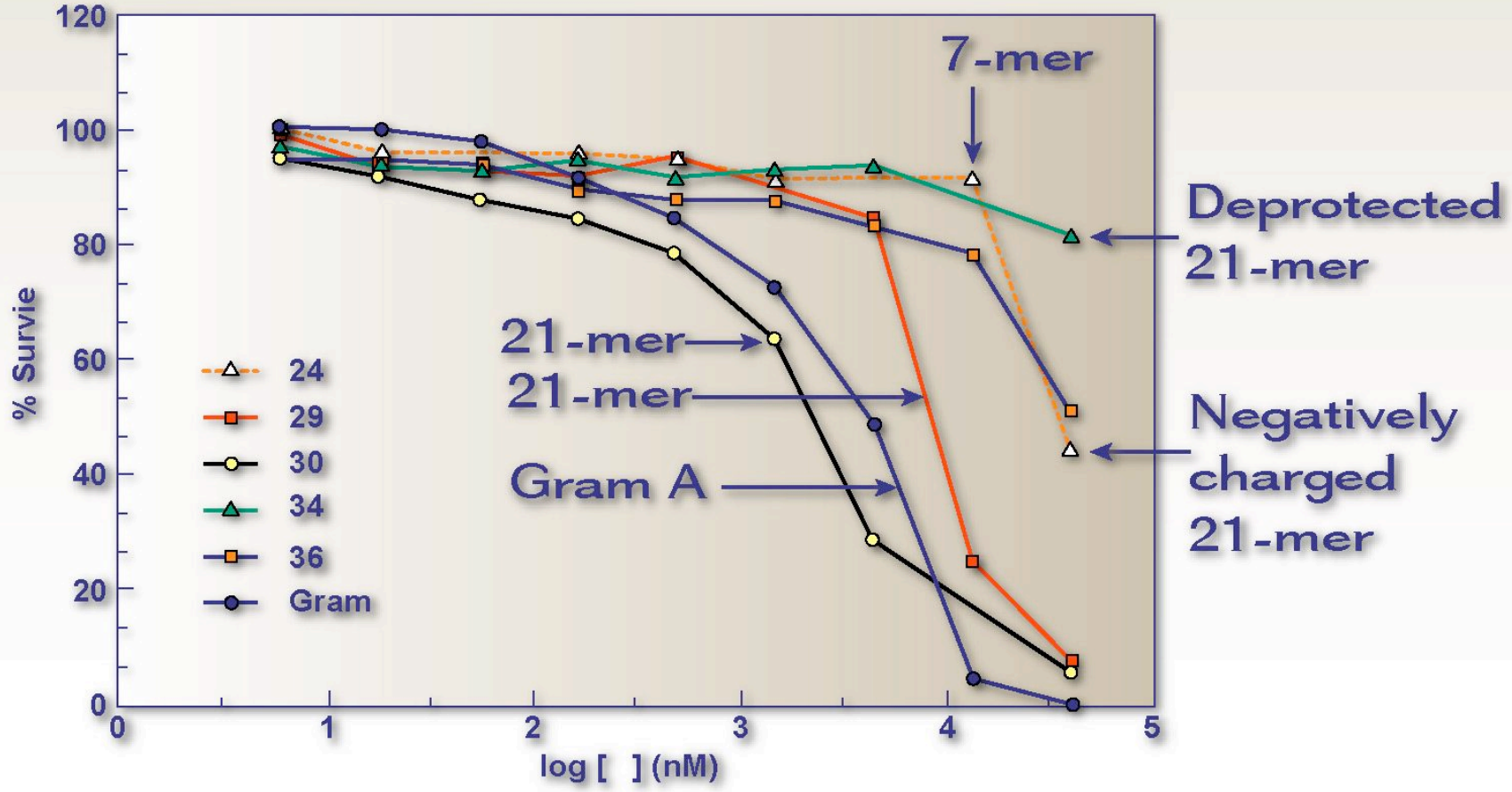
↓

No possibility to develop resistance





Cytotoxicity Towards P388 Cancer Cells



New Approach Towards Sensing

- Develop a simple and general assay for a wide range of biologically relevant analytes

→ Ultimately to the single molecule level

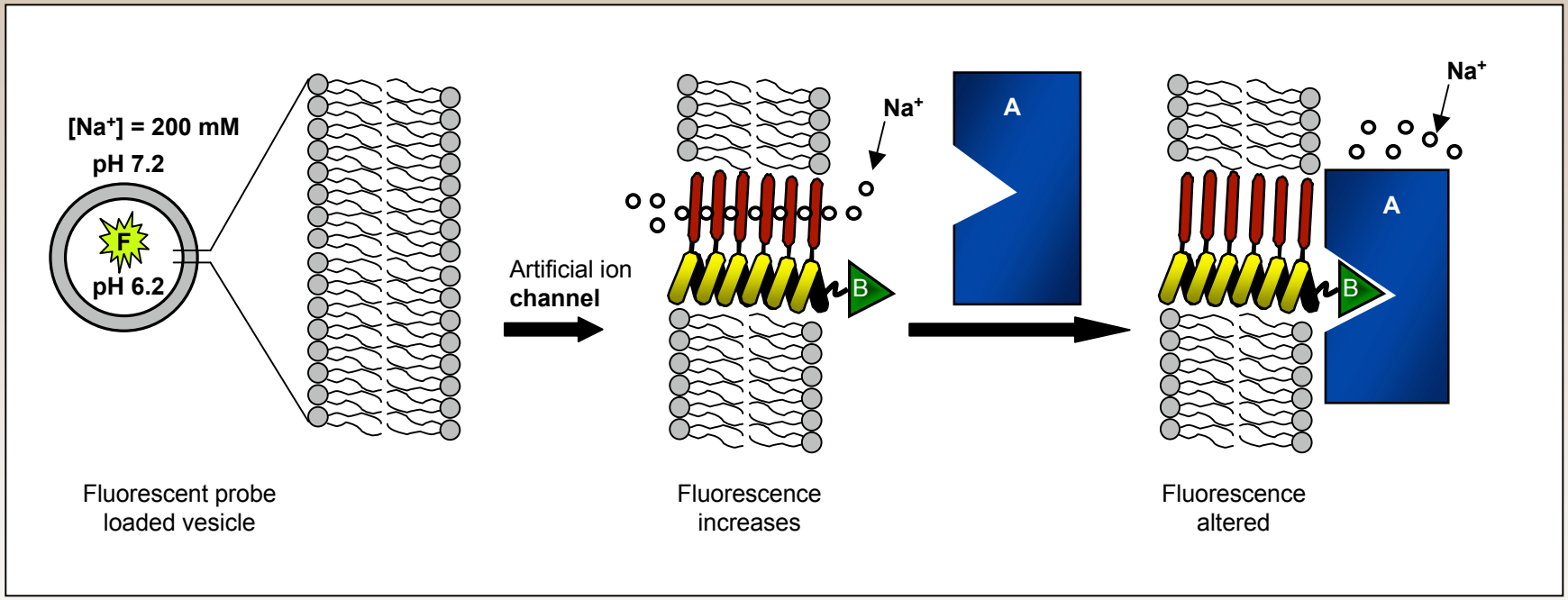
New Approach Towards Sensing

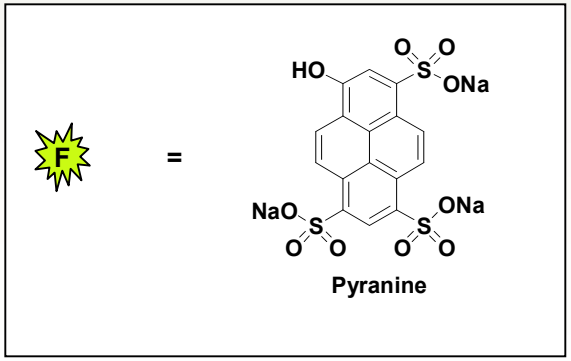
- Develop a simple and general assay for a wide range of biologically relevant analytes

→ Ultimately to the single molecule level

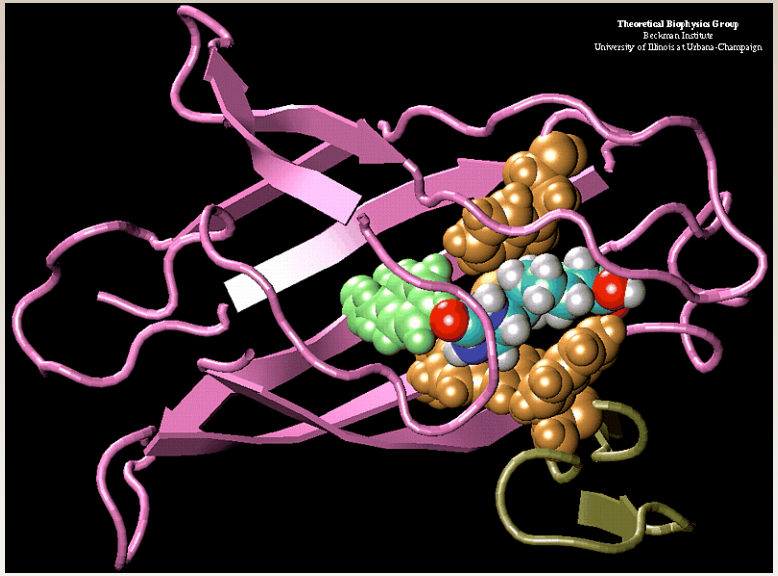
- Exploit the ion transport properties of peptide nanostructures
- Exploit first the versatility of fluorescence spectroscopy

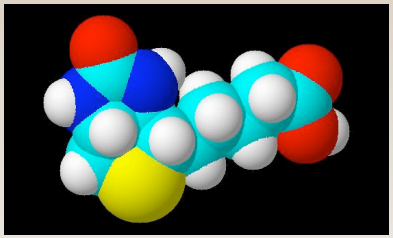
Working Hypothesis





Avidin~Biotin System





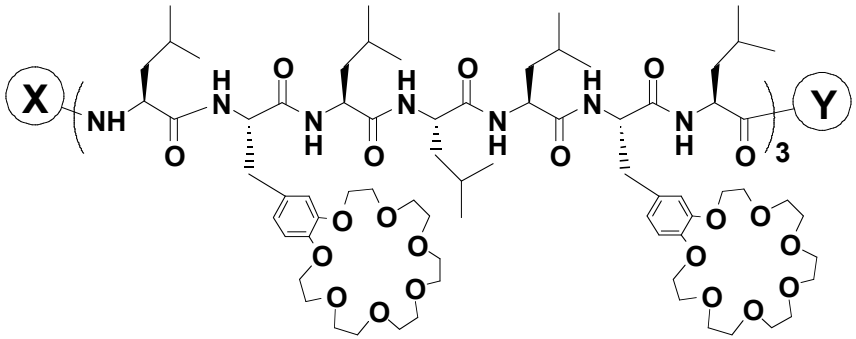
Biotin : MW = 244.31

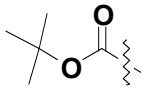
Avidin : MW = ~67 kDa

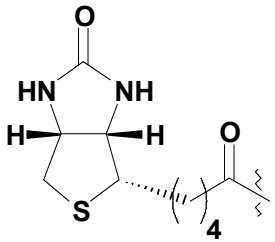
Monomer contains 128 amino acid residues

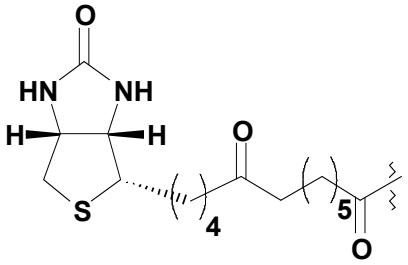
$K_a = 10^{15}\ M^{-1}$

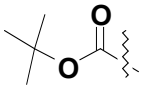
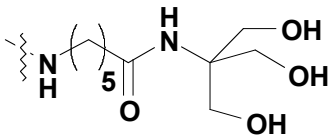
Biotinylated Nanostructures



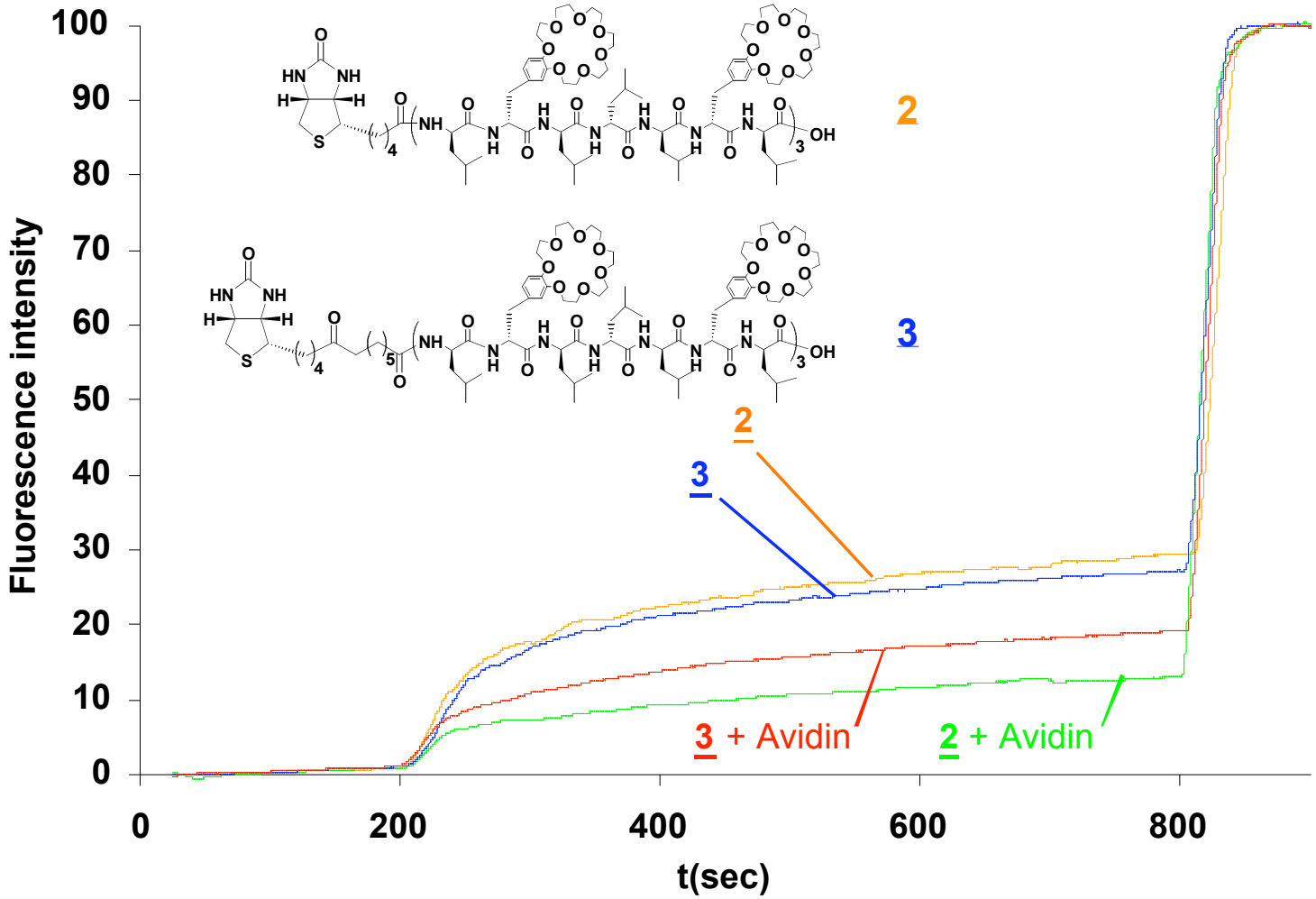
1 X =  Y = OMe

2 X =  Y = OH

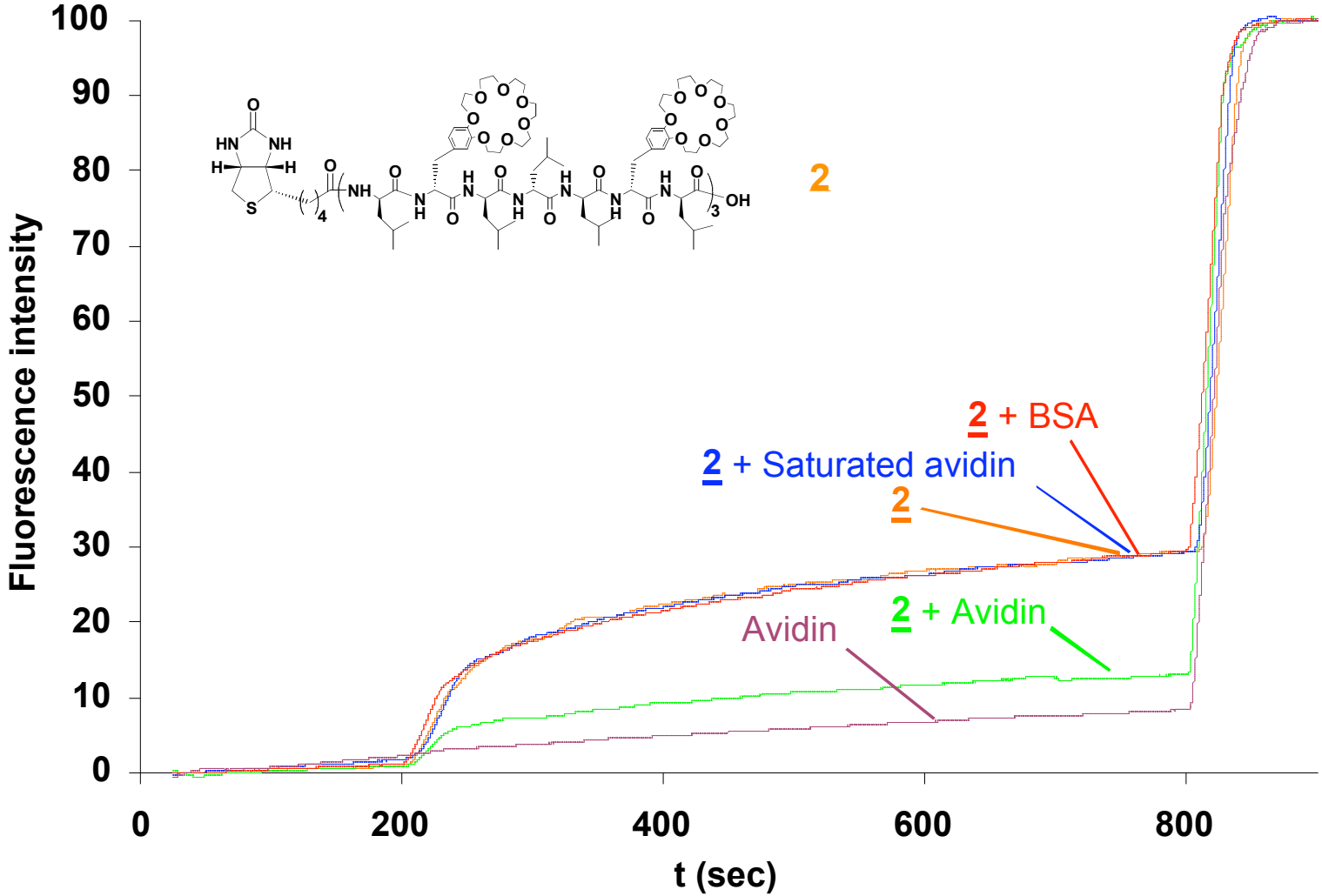
3 X =  Y = OH

4 X =  Y = 

Fluorescence Studies



Control Experiments



Conclusions

- Novel and general approach to artificial ion channels
- Compounds adopt the predicted α-helix conformation in TFE and in membranes
- Exist as monomers in low polarity environment

Conclusions (Part 2)

- Function as true artificial ion channels in vesicles and in bilayer membranes
- Ions travel by the channel formed by the crown ethers alignment
- Cytotoxic to cancer cells

Conclusions

- Developed a fluorescent assay using peptide nanostructures as « molecular transducers »
- Simple and applicable to a wide range of clinically important label-free analytes

Acknowledgments

Graduate Students

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G. Dewynter (Montpellier)
R. Gaudreault (LavaL)

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R. Barratin

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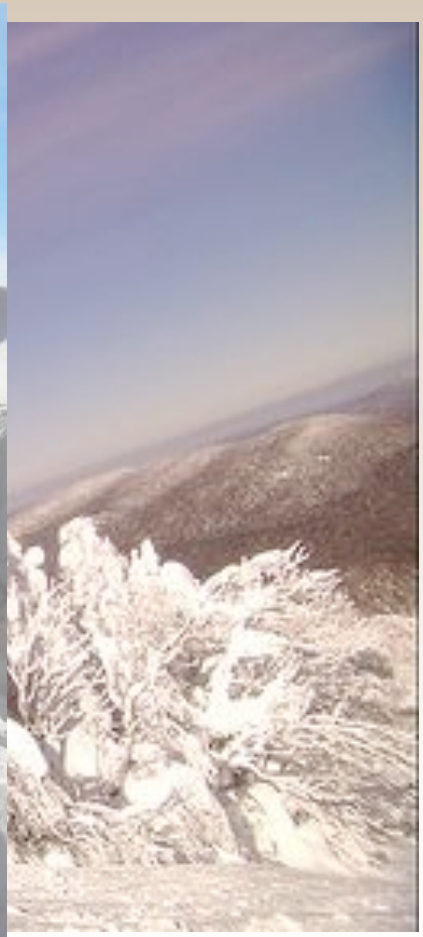
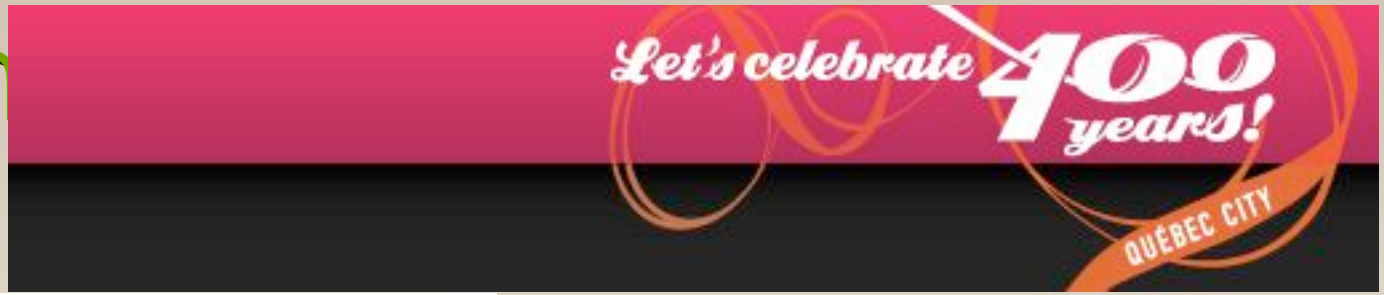
- NanoQuébec
- FQRNT Gouvernement du Québec
- FRSQ Gouvernement du Québec
- Université Laval
- Ministères de l'enseignement supérieur France/Québec

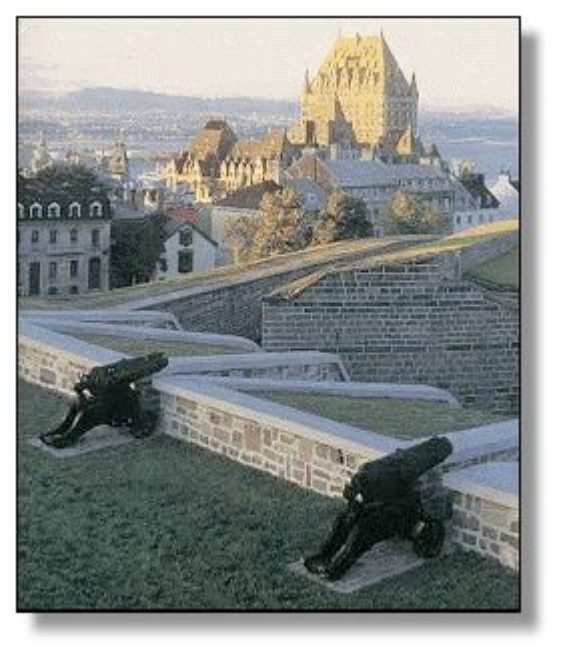
The Québec Advantage*

8 Months without air conditioning needs



2008 Québec City 400th Ar





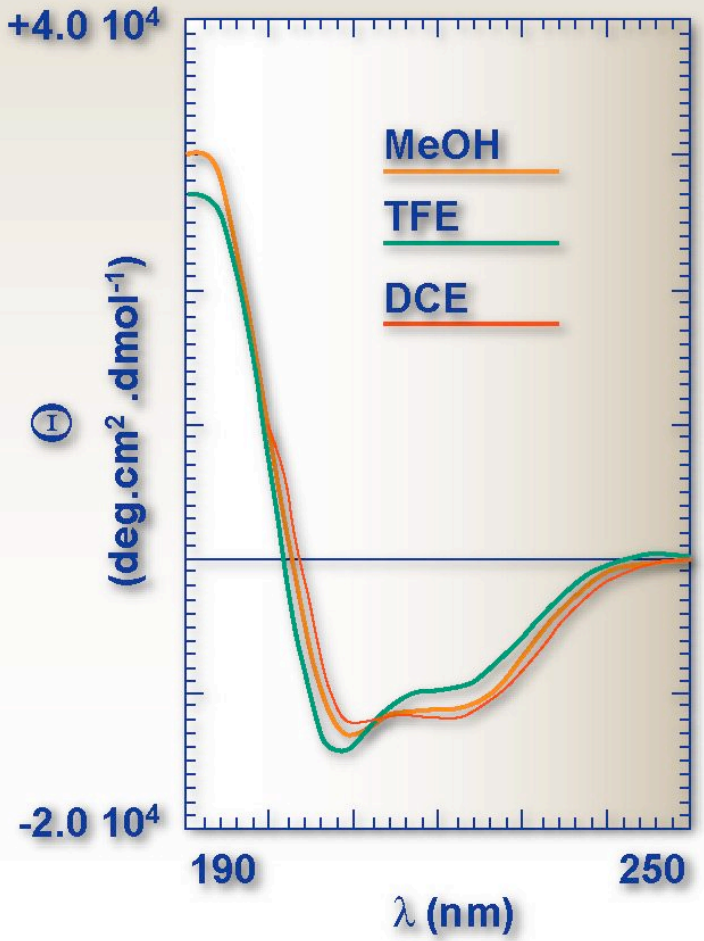
Thank you!

Comparison

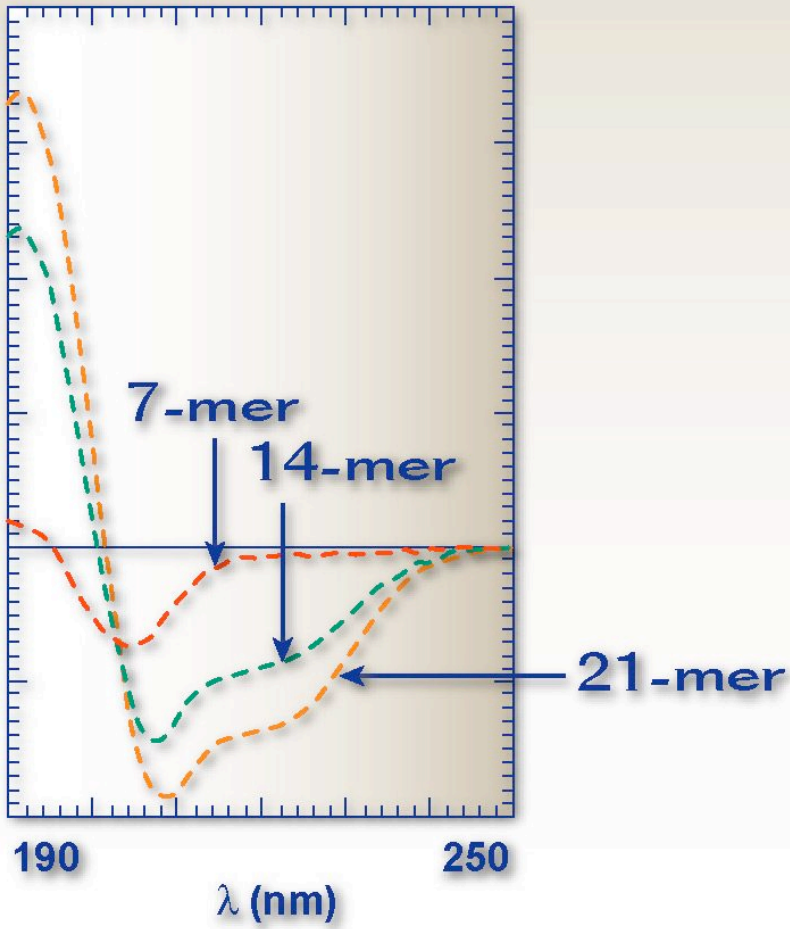
	21-Crown-7 channel	18-Crown-6 channel
Functional	Yes	Yes
Conductance	2.5 and 5 pA	3.5 pA
Lifetime	>1 sec	100-200 msec

Circular Dichroism Studies

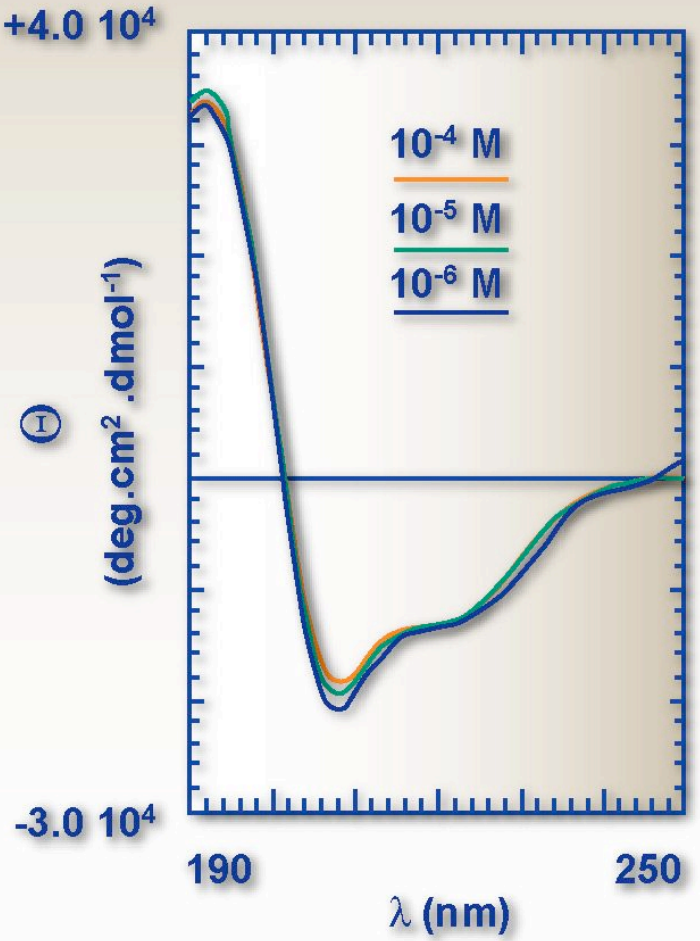
Different solvents...

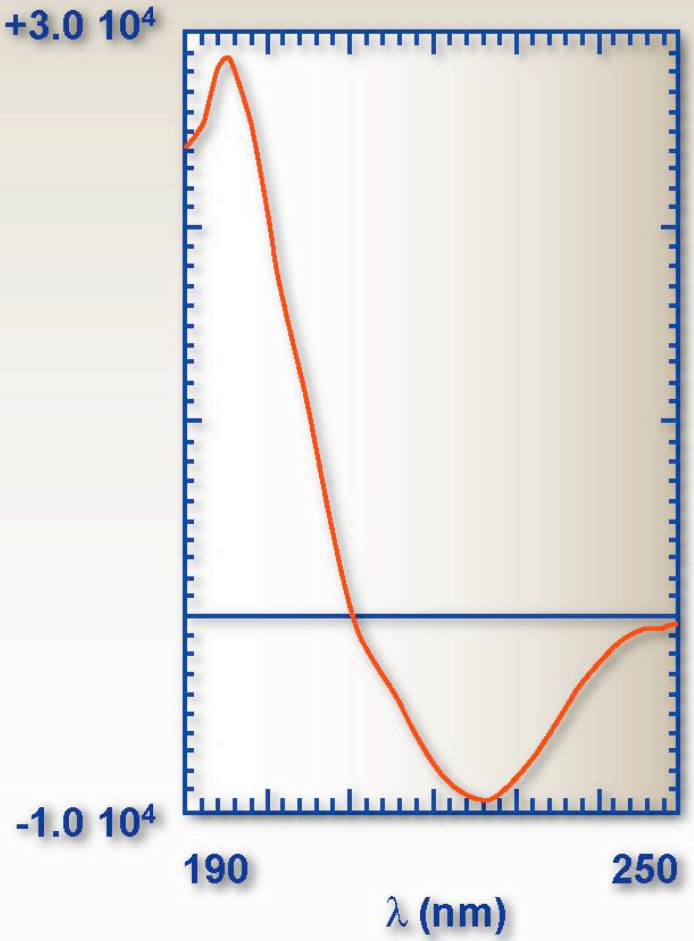


Different lenghts...



CD in Vesicles

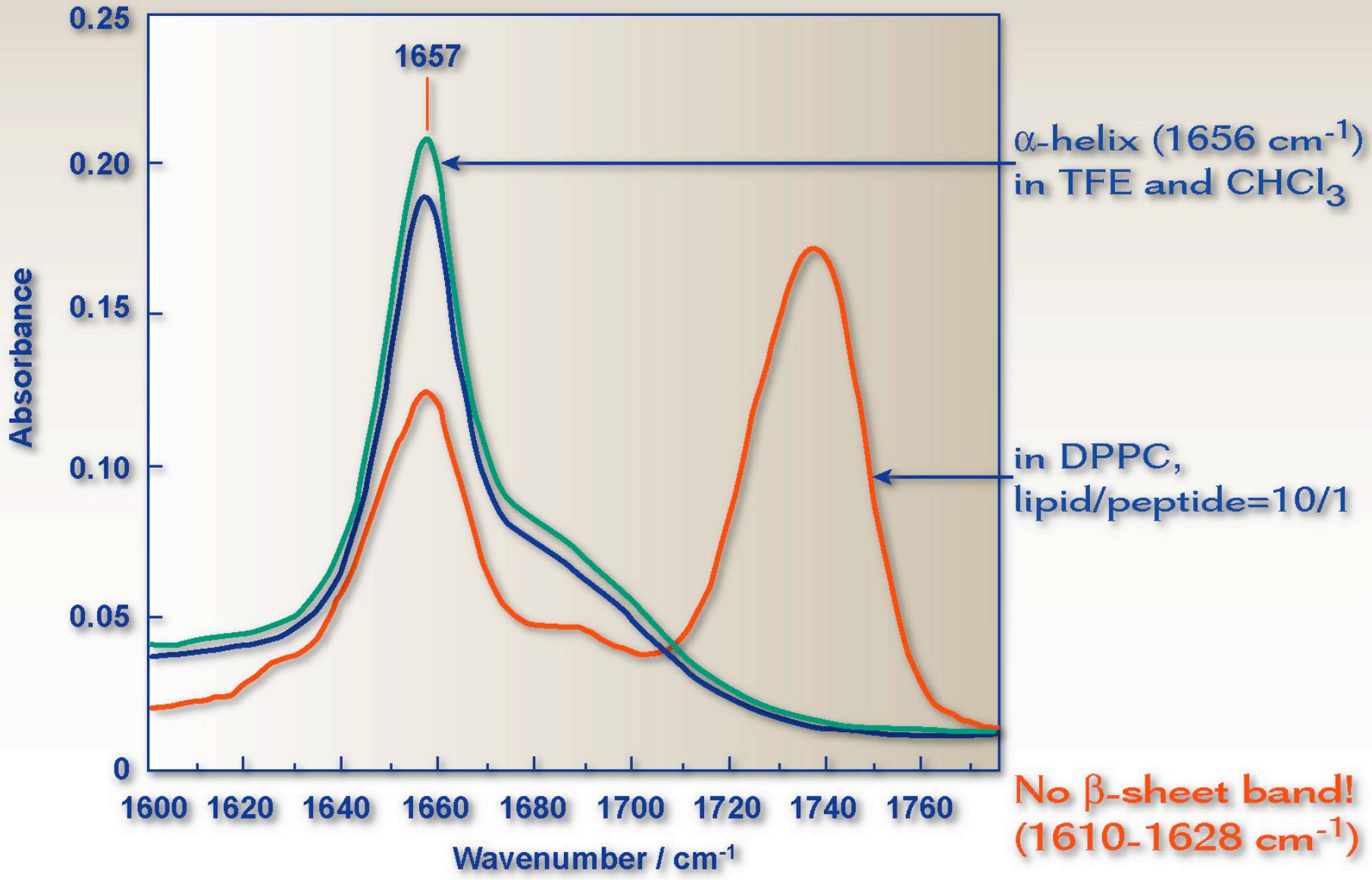




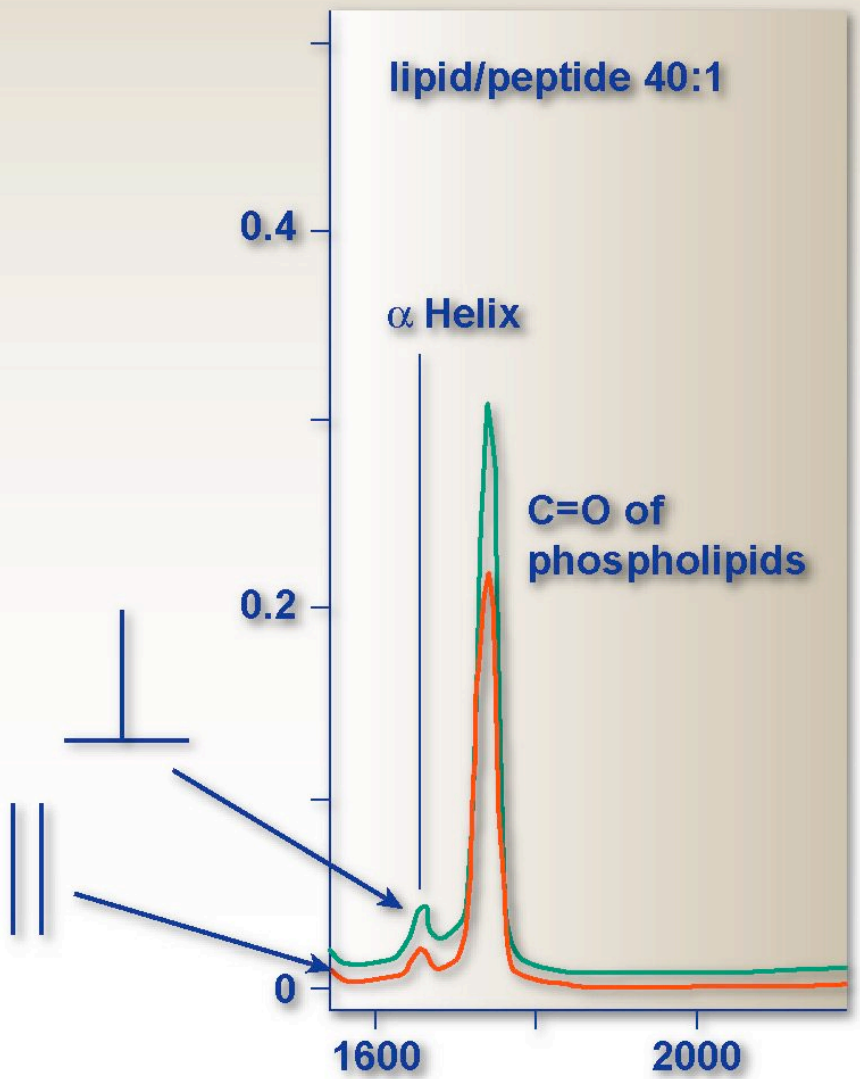
[ ]= 10^{-5} - 10^{-3} M in TFE

EYL Vesicles lipid/peptide=80/1

FTIR Spectroscopy



ATR Studies



Using polarized ATR spectroscopy, the angle θ between a helix and the bilayer normal can be found...

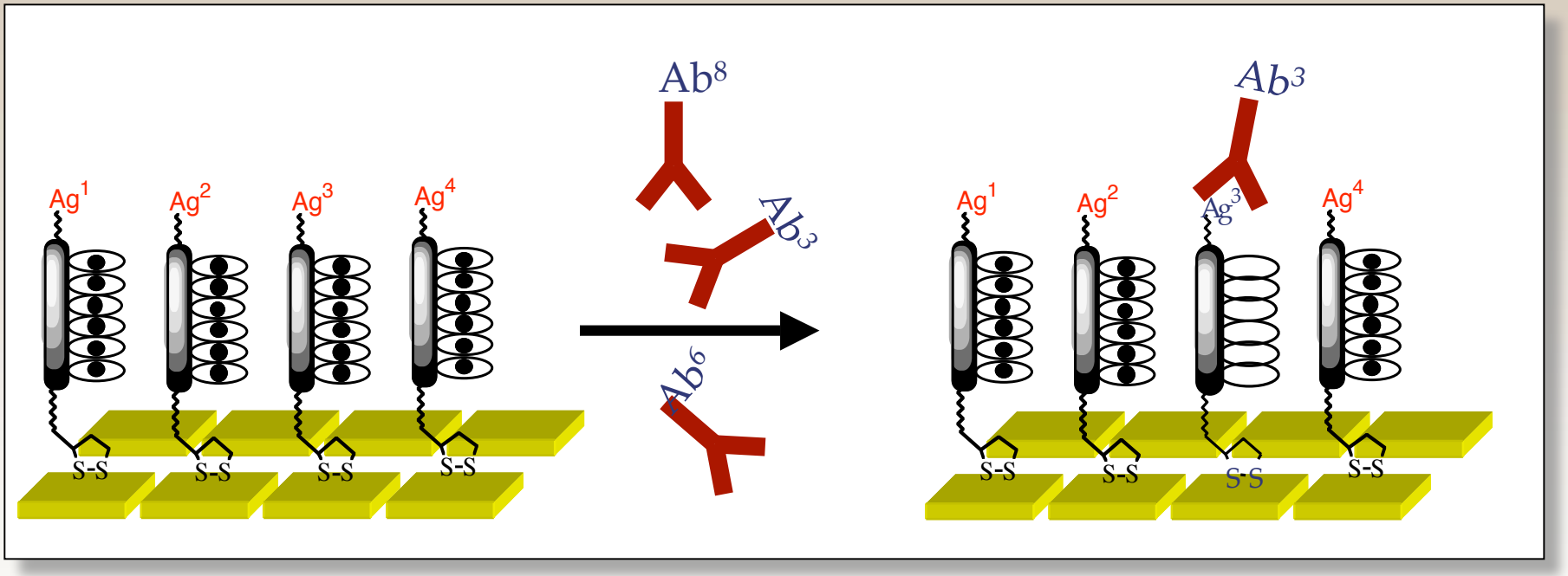
$$f(\theta) = \frac{R - 2}{R + 1.45} \; \frac{2}{3\cos^2\gamma - 1}$$

$$f(\theta) = \frac{3\cos^2\theta - 1}{2}$$

ATR Results

Samples	Spectral bands: V_{CH2} sym R	V_{CH2} sym θ	$V_{amide\ 1}$ R	$V_{amide\ 1}$ θ
DMPC	1.09±0.01	25.5±0.1		
BOC-21 mer (21-C-7)-)Me 1	1.14±0.01	28.3±0.2	1.84±0.02	58.7±0.2
BOC-21 mer (18-C-6)-OMe 2	1.14±0.01	28.3±0.2	1.76±0.20	59.4±1.2
BOC-21 mer (13-C-4)-OMe 3	1.18±0.02	29.9±1.0	2.01±0.05	54.6±0.9
BOC-21 mer (13-C-4)-OH 4	1.17±0.02	29.5±1.0	1.86±0.02	57.5±0.3
H-21 mer (13-C-4)-OH 5	1.29±0.01	34.3±0.3	1.84±0.01	57.6±0.1

Towards Microarray Nanosensors



Gold patterned microarray

Simultanous detection of analytes at single molecule level