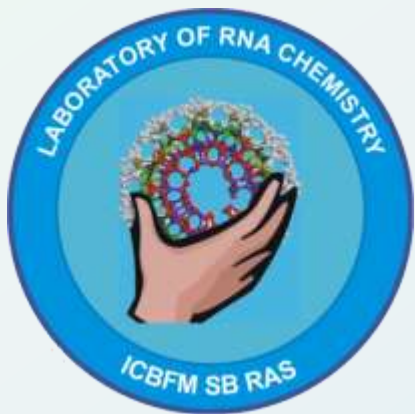


ATTACHMENT OF NUCLEIC ACID FRAGMENTS ONTO SWNT SURFACE

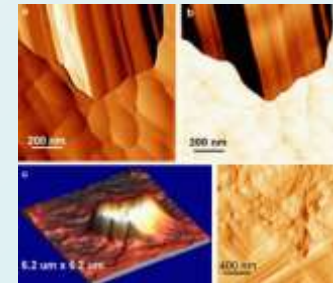
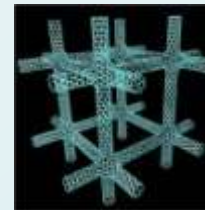
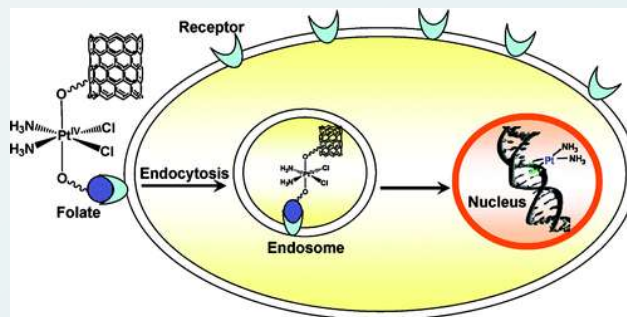
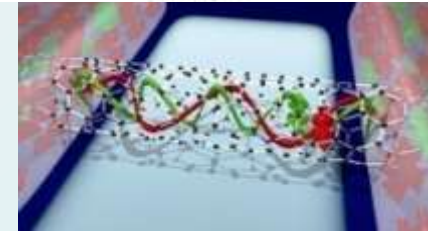
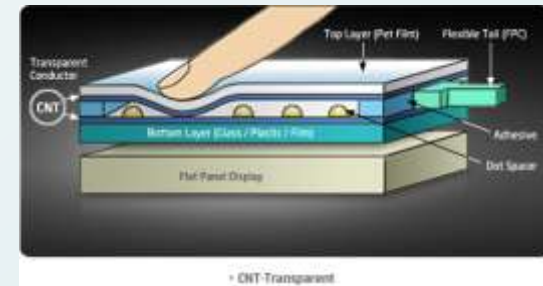
Evgeny K. Apartsin, Marina Yu. Buyanova,
Daria S. Novopashina

*Institute of Chemical Biology and Fundamental Medicine
Novosibirsk, Russia
e-mail: eka@niboch.nsc.ru*



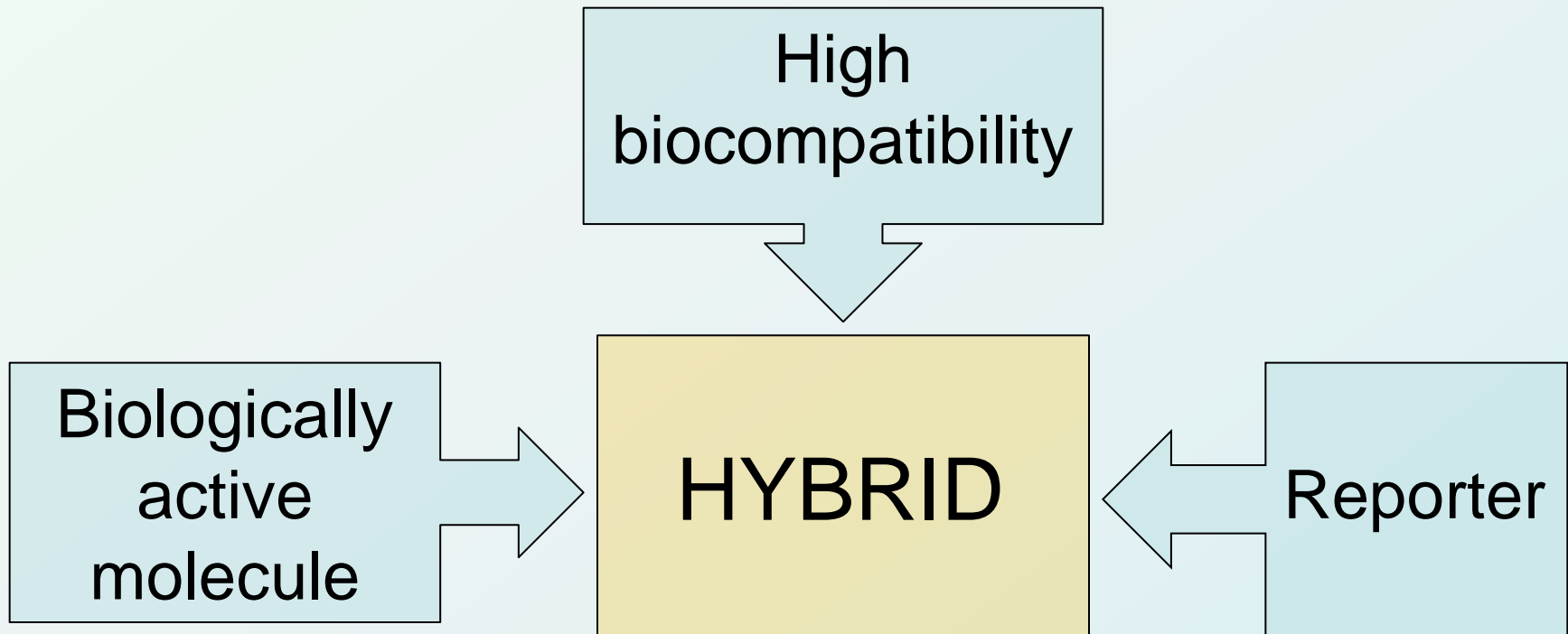
Various applications of carbon nanotubes

- Energy storage
- Nanocatalysts
- Nanosensors/**biosensors**
- Nanocomposites
- Nanoarchitecture
- **Drug delivery**

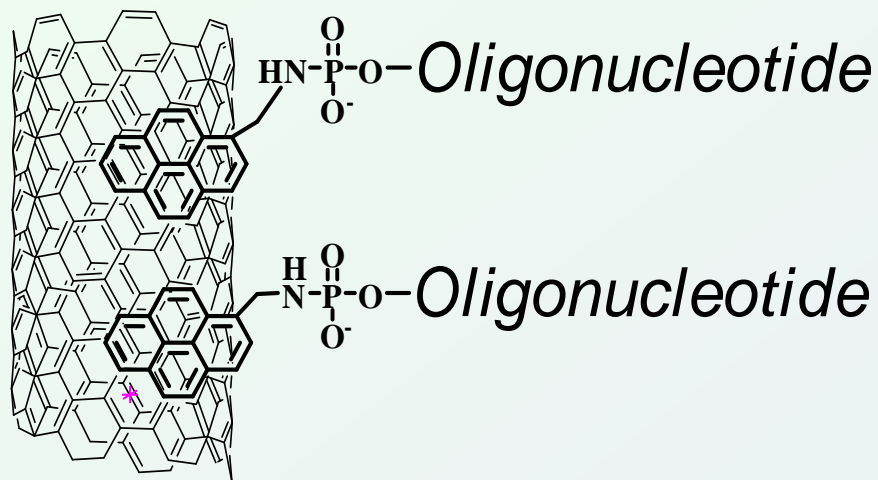


The aim:

Elaboration of a method to obtain multifunctional hybrids of carbon nanotubes with nucleic acids fragments and their complexes



CNT-NA hybrids: novel approach for preparation



High density of functionalization

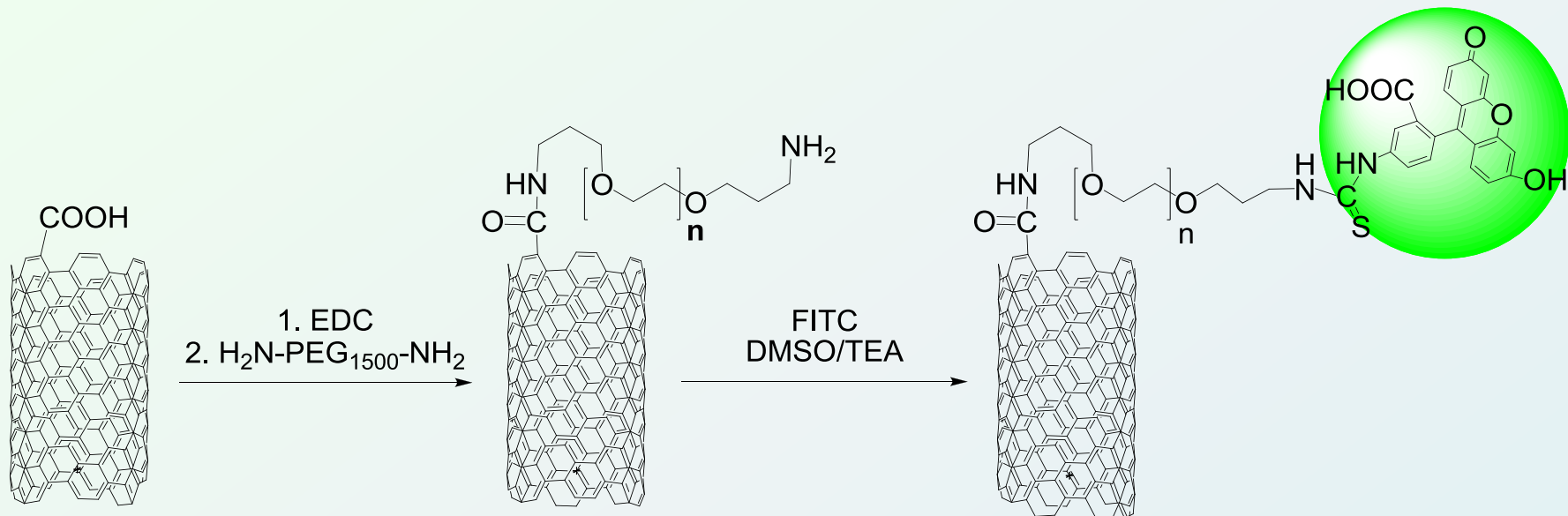
Mild conditions of immobilization

Simple procedure of oligonucleotide derivatives synthesis

Strong immobilization by pi-pi-interactions of aromatic systems

Possibility to construct multifunctional CNT

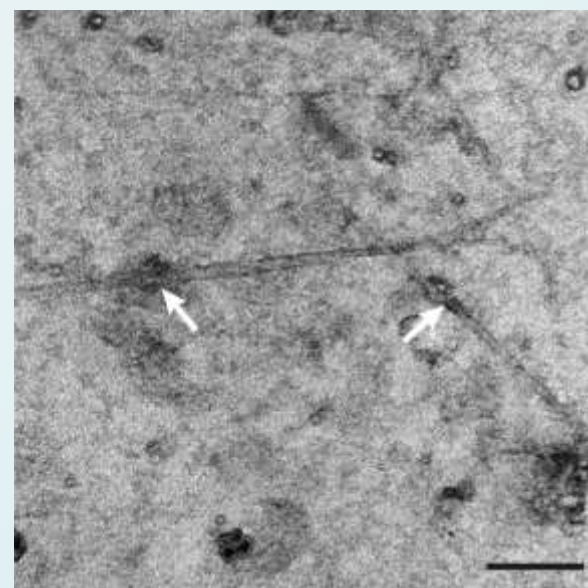
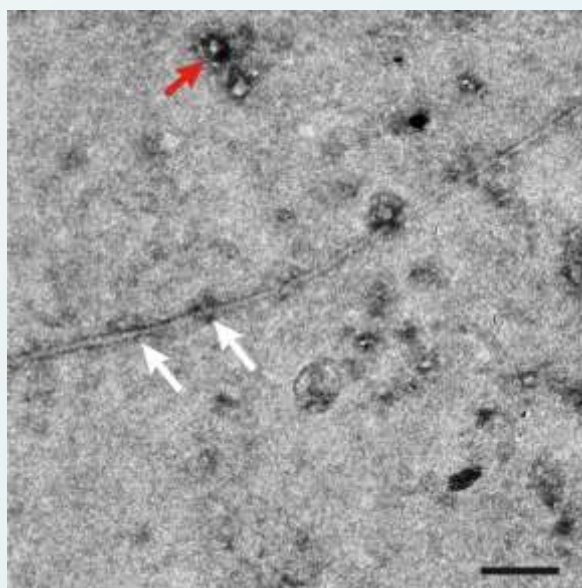
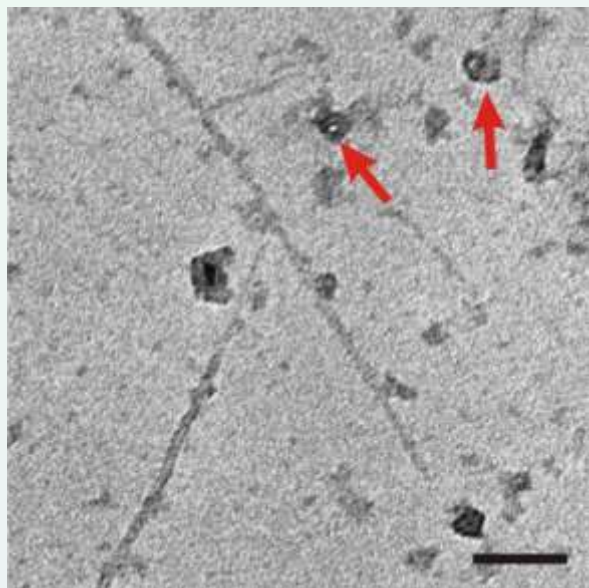
Functionalized SWNTs



SWNT-COOH

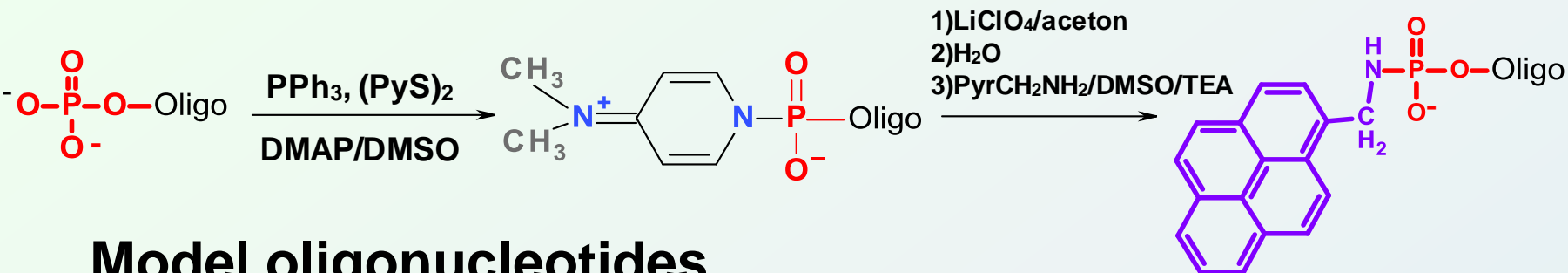
SWNT-PEG

SWNT-PEG-FITC



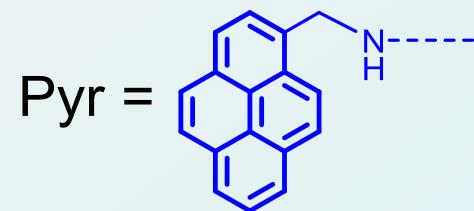
Scale bar – 100 nm

Pyrene conjugates of oligonucleotides

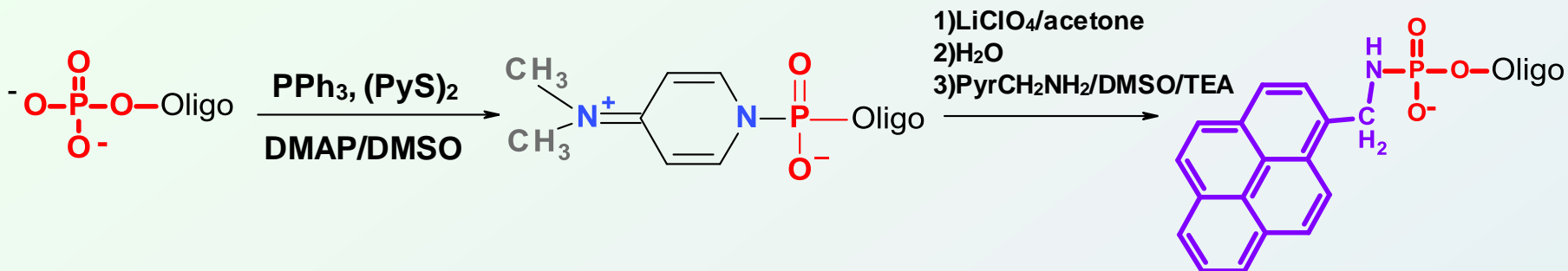


Model oligonucleotides

| Conjugate | Molecular weight | |
|------------------------------|------------------|-------------------------|
| | Calculated | Found (MALDI TOF MS) |
| 5'-PyrpA(pA) ₉ | 3523,35 | 3522.24 |
| 5'-PyrpA(pA) ₁₄ | 5169,38 | 5169.94 |
| 5'-PyrpA(pA) ₁₉ | 6815,41 | 6819.67 |
| 5'-PyrpA(pA) ₂₄ | 8461,44 | 8467.08 |
| 5'-PyrpU(pU) ₁₄ | 4823,78 | 4824.88 |
| 5'-PyrpU(pU) ₁₉ | 6354,61 | 6361.1 |
| 5'-PyrpU(pU) ₂₄ | 7885,44 | 7891.73 |
| 5'-PyrpdA(pdA) ₁₄ | 4929,39 | 4929.79 |
| 5'-PyrpdA(pdA) ₁₉ | 6495,43 | 6510.31 |
| 5'-PyrpdA(pdA) ₂₄ | 8061,46 | 8103.20 |
| 5'-PyrpT(pT) ₁₄ | 4794,19 | 4795.28 |
| 5'-PyrpT(pT) ₁₉ | 6315,16 | 6318.1 |
| 5'-PyrpT(pT) ₂₄ | 7836,12 | 7844.39 |

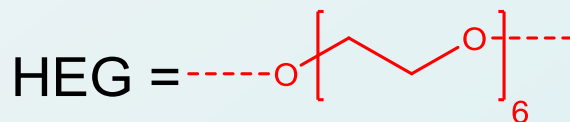
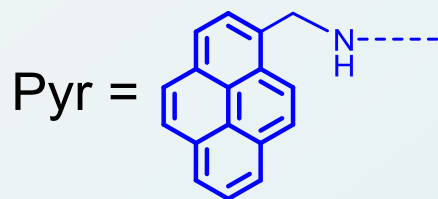


Pyrene conjugates of oligonucleotides

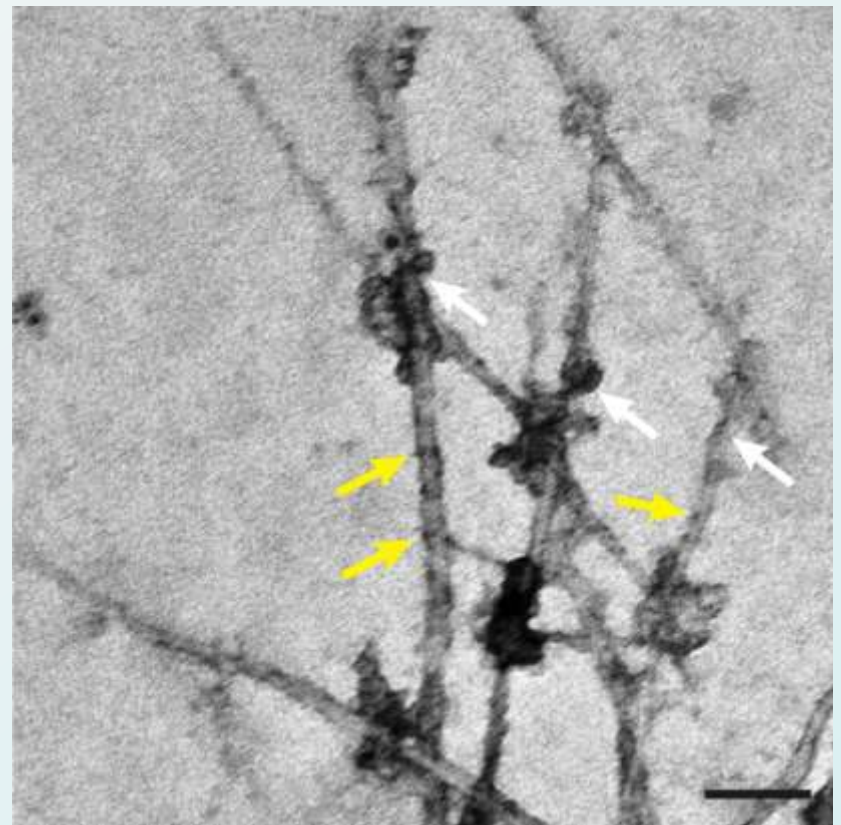
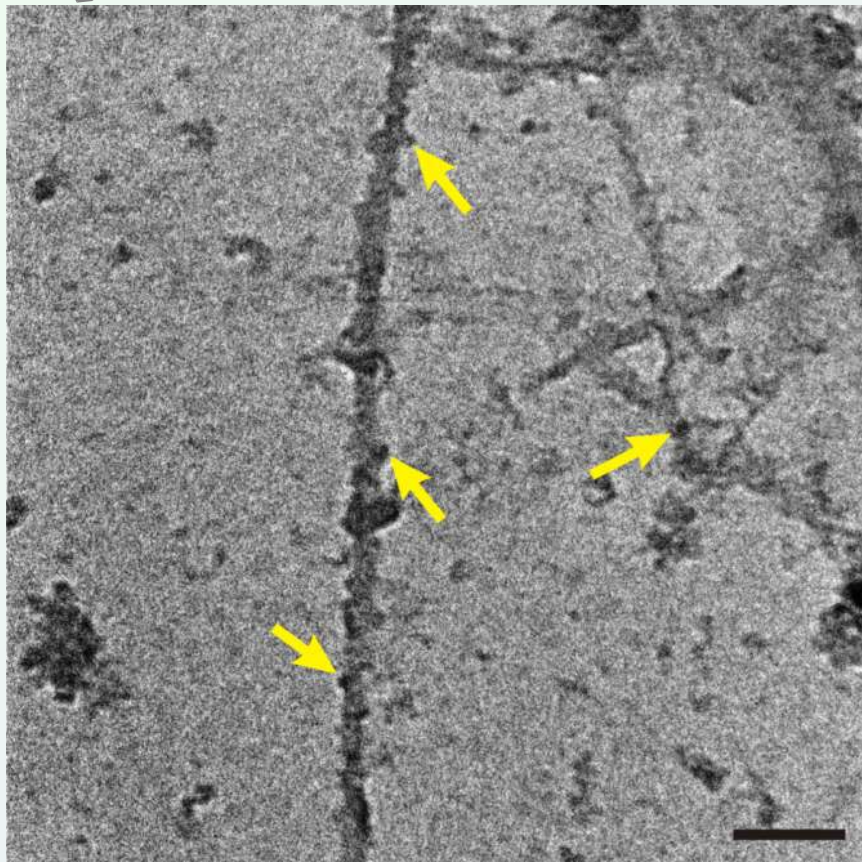
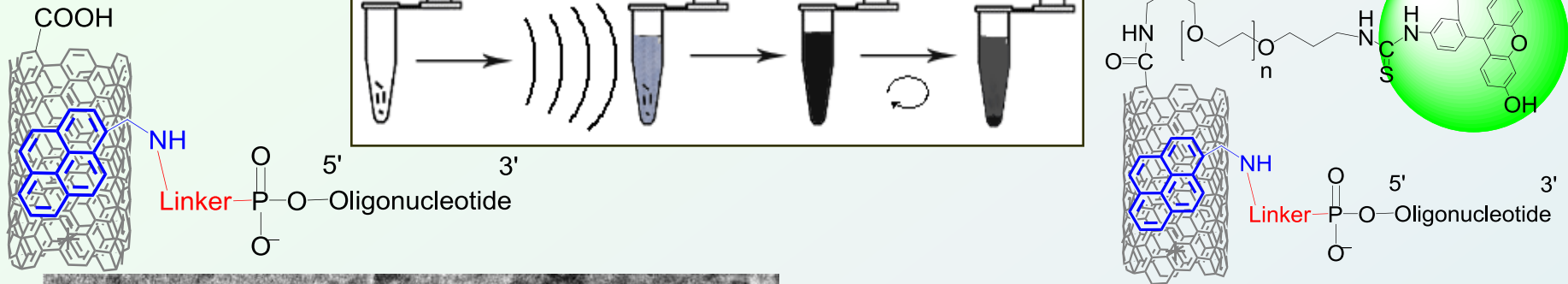


Components of therapeutic NA-constructions

| Abbreviation | Conjugate | Molecular weight | |
|-----------------|--------------------------------------|------------------|----------------------|
| | | Calculated | Found (MALDI TOF MS) |
| PyrHEG22 | 5'-PyrpHEGpd(ACCCTGAAGTTCCGGCAAGCTG) | 7357.92 | 7364.05 |
| Pyr22 | 5'-Pyrpd(ACCCTGAAGTTCCGGCAAGCTG) | 7013.61 | 7016.89 |
| PyrHEG17 | 5'-PyrpHEGpd(AACCGTGGTCATGCTCC) | 5783.90 | 5785.11 |
| Pyr17 | 5'-Pyrpd(AACCGTGGTCATGCTCC) | 5439.64 | 5448.74 |
| Pyr21 | 5'-PyrpAUUAACAACUUGUCAAGCCAA | 6941,30 | 6944.94 |
| PyrHEG21 | 5'-PyrpHEGpAUUAACAACUUGUCAAGCCAA | 7285,59 | 7290.98 |



SWNT/oligonucleotide hybrids

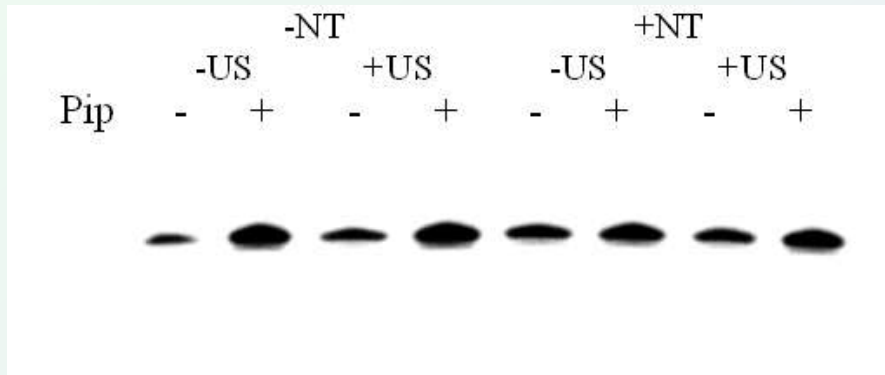


Scale bar – 100 nm

Stability of oligonucleotides at the hybrid formation conditions

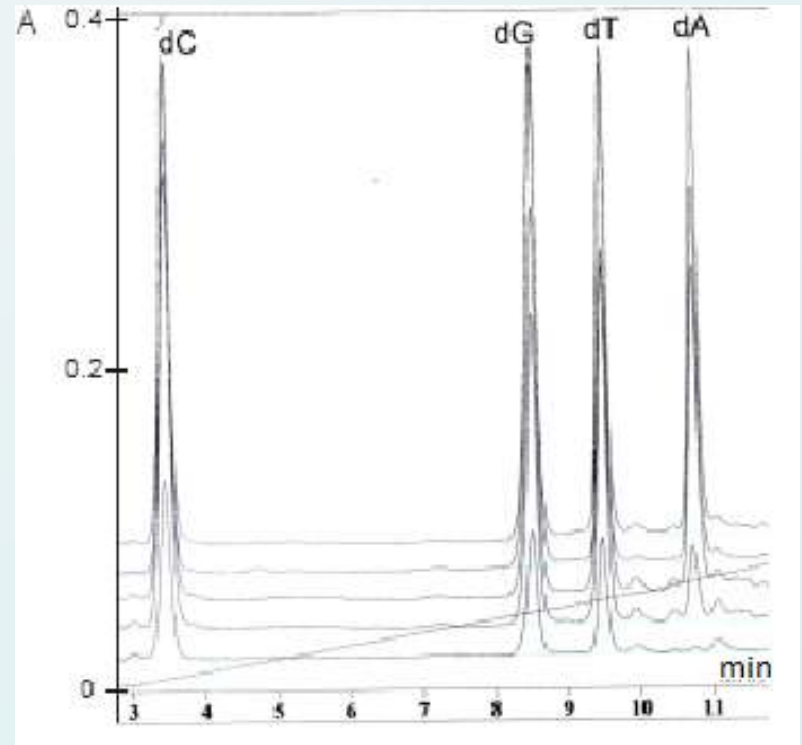
AP-sites and strand breaks test

1. Sonication of oligonucleotide with and without SWNT;
2. Piperidine treatment;
3. PAGE assay.

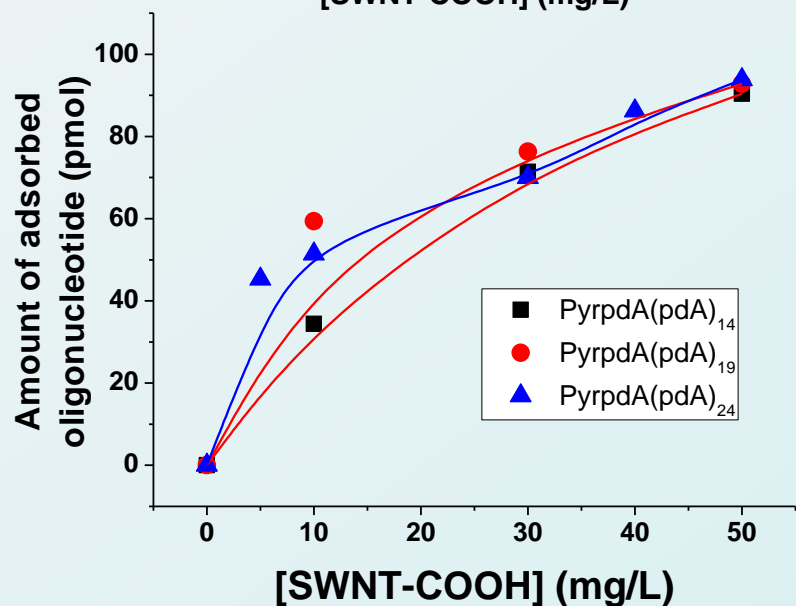
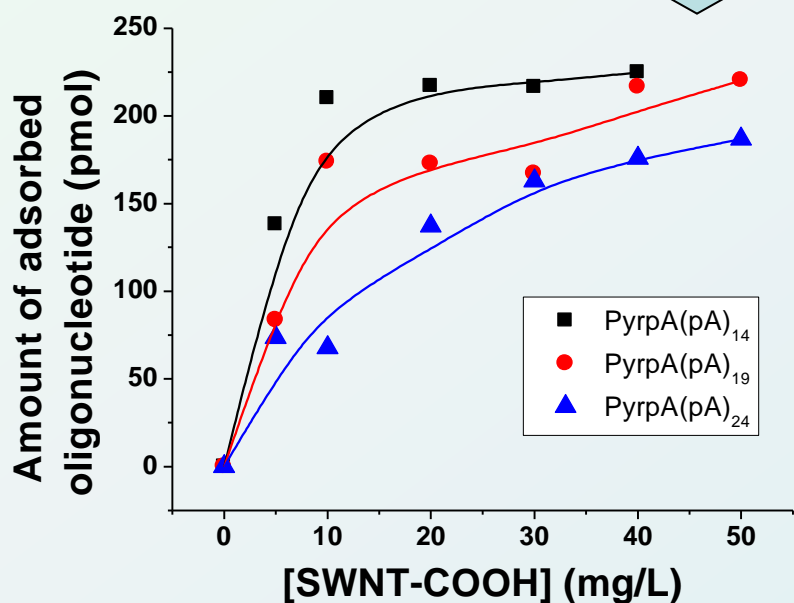
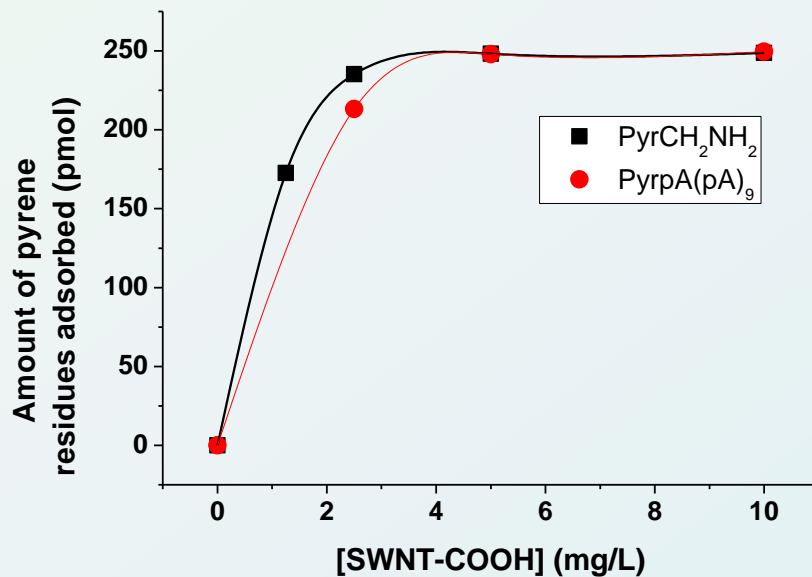
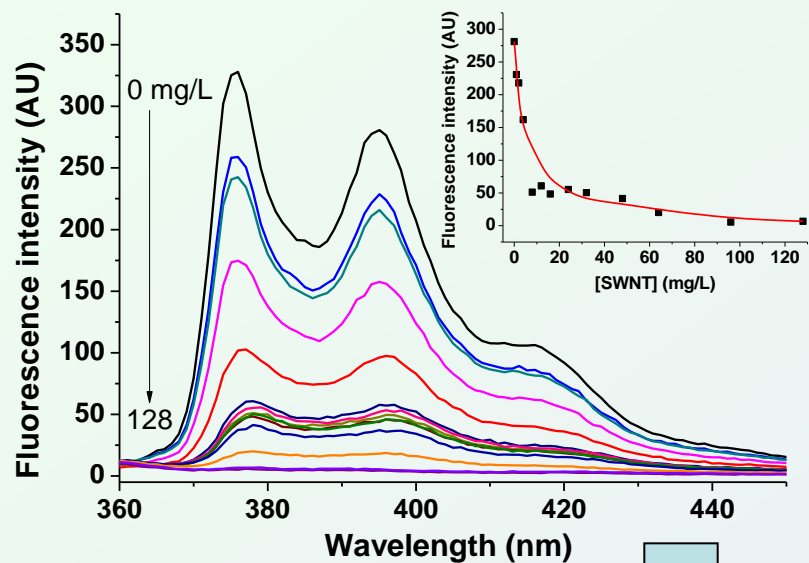


Nitrogenous bases oxidation test

1. Sonication of oligonucleotide with and without SWNT;
2. Venom phosphodiesterase hydrolysis;
3. HPLC of reaction mixture.

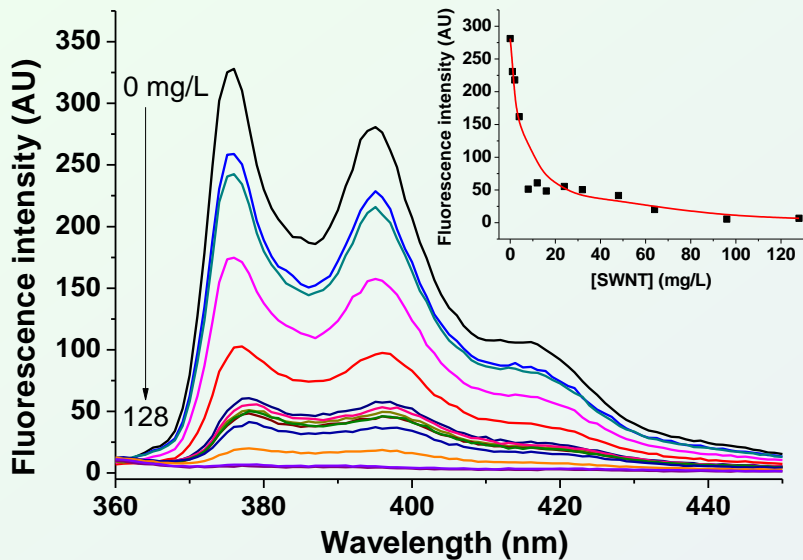


Adsorption of oligonucleotides on SWNT surface

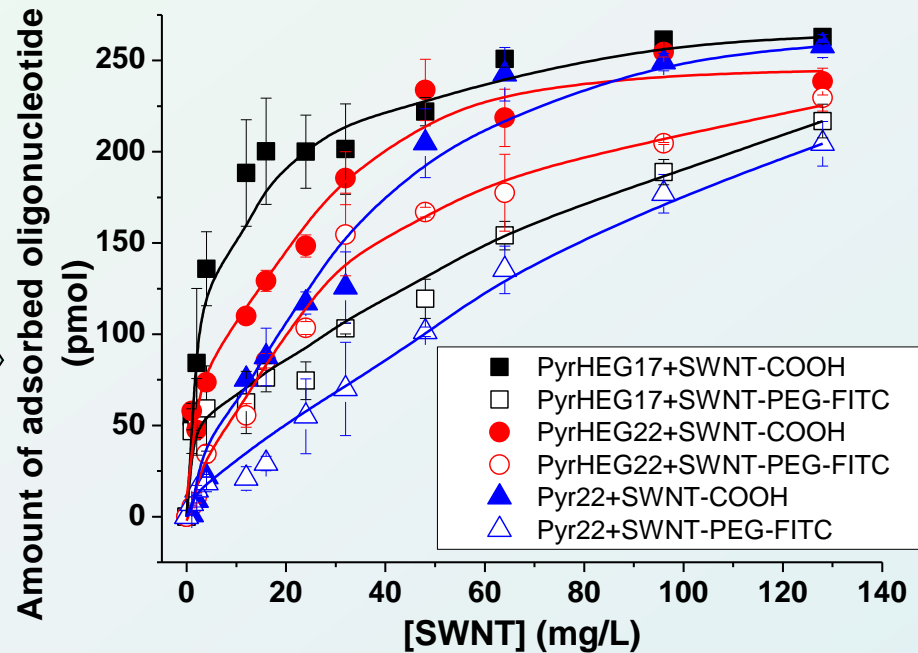


Conditions: 0.01M Tris-HCl (pH 7.5), 0.1M NaCl, 1mM Na₂EDTA, 25 °C, λ_{ex} 345 nm, oligonucleotide concentration 1 μ M.

Adsorption of oligonucleotides on SWNT surface



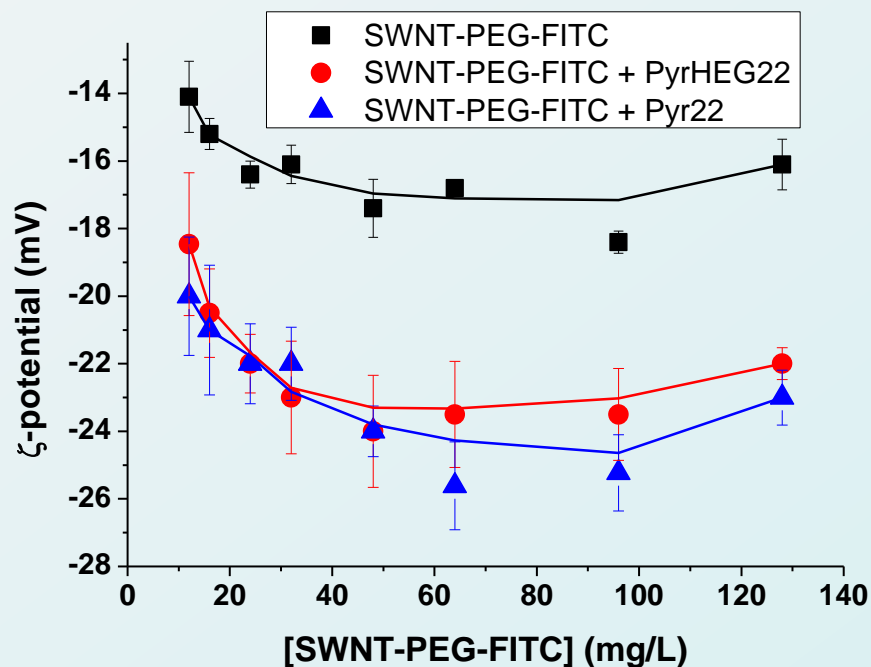
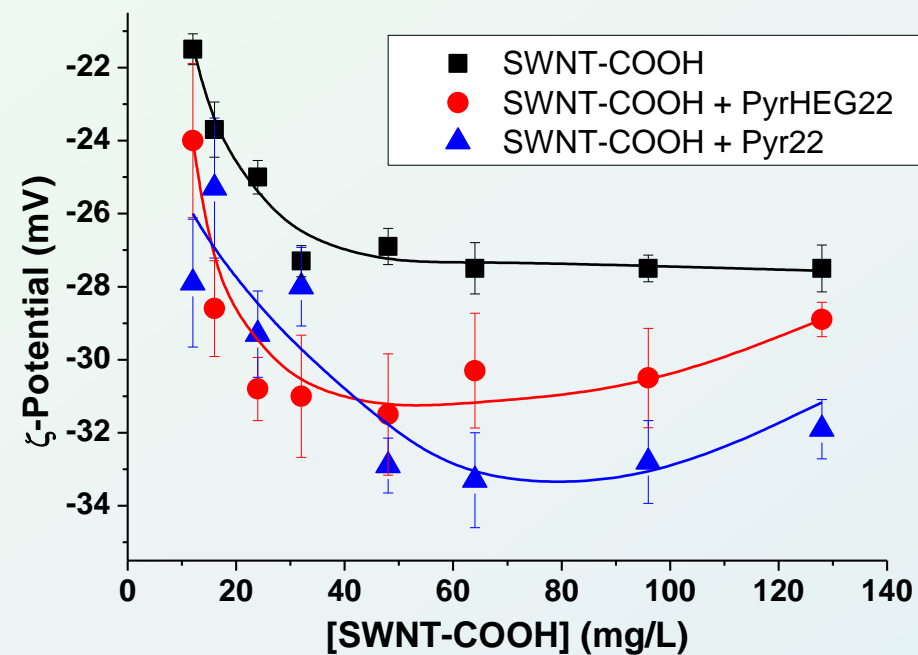
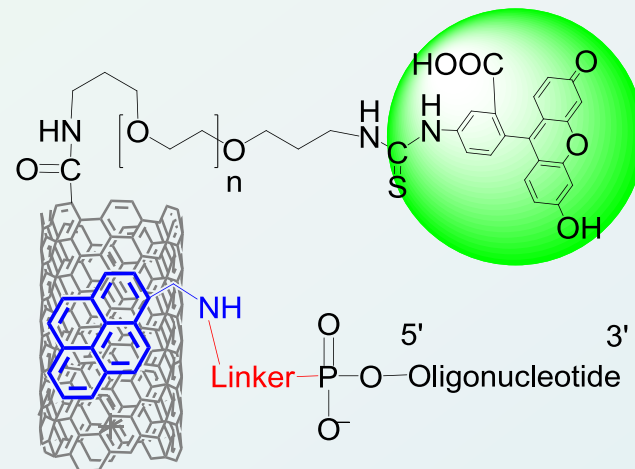
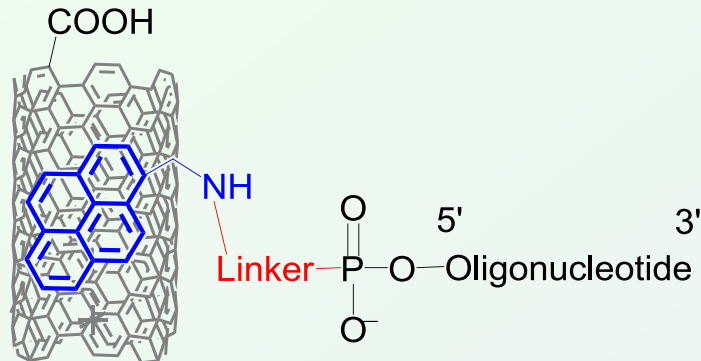
Conditions: 0.01M Tris-HCl (pH 7.5), 0.1M NaCl, 1mM Na₂EDTA, 25 °C, λ_{ex} 345 nm, oligonucleotide concentration 1 μ M.



| Parameter | Effect on adsorption efficacy |
|--|-------------------------------|
| PEGylation of SWNT | ↓↓ |
| Increasing of oligonucleotide length | ↓ |
| Presence of HEG linker at 5'-terminus of oligonucleotide | ↑ |

SWNT capacity: 20-100 μ mol/g depending on the oligonucleotide structure and SWNT functionalization

Hydrodynamic properties of hybrids

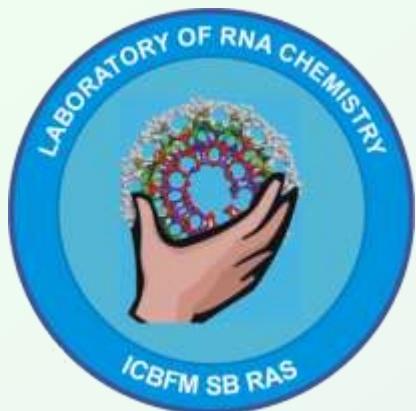


Conditions: 0.01M Tris-HCl (pH 7.5), 0.1M NaCl, 1mM Na₂EDTA, 25 °C, oligonucleotide concentration 1 μ M.

Conclusion

- ❑ The novel type of multifunctional hybrids of oligonucleotides and single-walled carbon nanotubes was designed;
- ❑ SWNT-NA hybrids were characterized by physico-chemical methods. The regularities of hybrids formation were examined

Acknowledgements



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