WP1.8. Controlling specific protein-protein interactions using nanoparticles (Cígler, Bouř)

Research aims

- To understand in detail the pharmacologically relevant interaction of NDs with FGFs.
- Identify binding domains in individual members of FGF protein family which are responsible for specific interaction with the ND surface.
- To ascertain the responsible binding domain, associated protein conformational changes, and the molecular basis of this interaction by combination of advanced analytical methods.
- To use ab initio and molecular dynamics simulations as well asFTIR and Raman spectroscopies for better understanding of the ND surface itself, i.e. chemical and morphological features which are responsible for the protein binding.
- To propose novel chemical structures, either NP or small molecule-based, efficiently regulating the FGF levels in the organism.
- To control protein-protein interactions directly using inorganic non-toxic nanoparticles and open up a pathway to radically new approach for a selective treatment of diseases using the nanoparticle chemistry

Research plan and methodology

Controlling the protein-protein interactions using nanoparticles requires a precise understanding of the binding process at the molecular and atomic level. Up until now, several mechanisms of the interaction have been proposed. They include the involvement of specific protein domains in the surface interaction and the interplay of local charge, hydrophobic and shape effects.

The contribution of these factors to the binding and changes in the molecular structure of the proteins upon interaction with ND surface will be analyzed by physico-chemical methods. Because x-ray crystallography cannot be used in solutions and for the heterogeneous ND/protein systems, spectroscopic methods including nuclear magnetic resonance (NMR)[1] and optical spectroscopies, such electronic absorption and circular dichroism,[2] infrared absorption (IR)[3] and Raman scattering will be used to obtain the desired information. In the project, we will concentrate on:

- (i) Spectroscopy of vibrational optical activity (VOA),
- (ii) NMR studies, and
- (iii) Mass spectrometry studies.

(i) VOA includes the vibrational circular dichroism (VCD) and Raman optical activity (ROA). It provides more resolved bands than the electronic methods, is more sensitive to fine variations in molecular structure including conformation, and can be modelled with a high accuracy by an initio methods.[4] In the past, we developed precise computational procedures to interpret ROA and VCD protein spectra in terms of structure and conformation.[5] These methods will we adapted to monitor the protein binding to the surfaces and complemented with multi-scale molecular mechanics/quantum mechanics simulations of the protein and ND surface behaviour.

(ii) Biomolecular NMR spectroscopy will also provide detailed information on conformational changes and region of the protein surface involved in the binding. First, we will obtain the comprehensive NMR resonance assignments and rapidly map the interaction interface by following the changes in NMR signal positions. Next, we will proceed with a detailed NMR structural characterization of the FGF conformational changes induced by binding for the selected systems

(iii) The mass spectrometry proteomic experiments will be based on gradual enzymatic digestion of the FGF interacting with ND and time-dependent analysis of the cleavage products (providing complementary information on binding to the other methods). The dynamic analysis of protein fragments will be performed using a newly purchased high-resolution mass spectrometer. The state-of-the-art instrumentation will make it possible to detect, characterize and quantify released peptides with high sensitivity and reliability.

All these three experimental strategies will be supported by quantum-chemical and molecular-dynamics modelling of ND surface as well as protein structure and conformational behaviour.

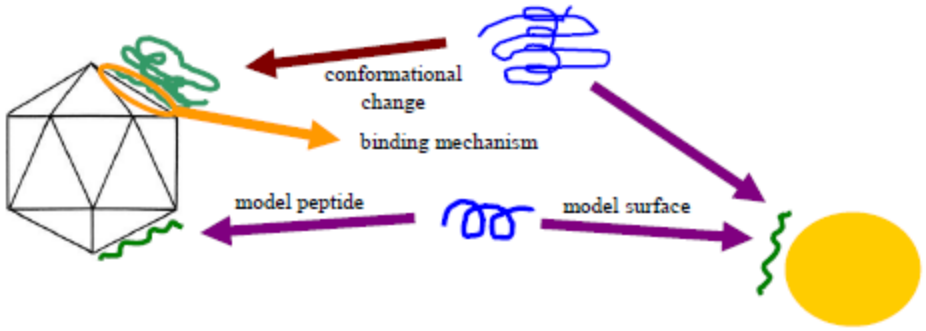

Fig. 2. To understand the high binding affinity of the protein to the nanodiamond surface, several aspects will be addressed. 1) Change of conformation of the protein upon binding will be monitored by CD, VCD, NMR and other spectroscopic methods, 2) suitable models will be proposed for the binding mechanism, 3) which can be verified using model peptide sequences or 4) model surfaces.

All the 22 members of the FGF family will be analysed. The proteins required in bigger quantities will be prepared by expression in bacteria and purified by standard techniques used in our laboratories. The proteins for NMR measurements will be isotopically labelled during the expression. To confirm the presence and location of specific binding domains, the strongest binders identified up to date (FGF1, 2, 8 and 10) will be structurally compared with the weaker ones (FGF 19, 21 and 22) and also with different growth factors (e.g. interleukin-1 and 6, interferon gamma). A simplified scheme of this approach is shown in Fig. 2. It includes the study of the conformational changes and binding mechanism followed by testing of the model peptides prepared to mimic the binding domains.

The surface chemistry of NDs will be also studied and tuned in order to strengthen the interactions with FGFs. FTIR and Raman spectroscopies will be used for understanding the surface structures on the ND surface which are responsible for the protein binding. The structure, surface density and individual contribution of the surface groups in the binding of either FGF proteins or model peptides will be ascertained (in collaboration with Treussart and Wolcott laboratories). Their molar ratio to other groups will be optimized. The influence of different chemical moieties on protein binding will be studied and various new chemical architectures on NDs (involving partial of full blocking the surface by polymers and de-novo attached moieties) will be synthesized. Based on these data, chemical structures optimal for the design of small molecule-based drugs regulating the FGF levels will be proposed, synthesized and tested. For purification and analyses of synthetic peptides, we will make extensive use of the UHPLC chromatography apparatus (Bouř group), which is a part of the proposed capital investment of this project. In collaboration with Krejčí's and Trantírek's laboratories, *in vitro* cell experiments and embryonic tests focused on localized treatment of cartilage-related diseases will be performed.

The project will be realized in a close collaboration with P. Řezáčová's Group (structure determination) and J. Cvačka's mass spectrometry (protein analysis) department using new high-resolution mass spectrometer, which is a part of the proposed capital investment of this project.

[1] A. G. Palmer, J. Williams, A. McDermott, *J. Phys. Chem.* **1996**, *100*, 13293-13310.
[2] W. C. Johnson, in *Molecular chirality in chemistry and life sciences, Vol. Book of abstracts*, CNR, Pisa, Italy, **1997**, p. 15.
[3] P. I. Haris, in *Infrared Analysis of Peptides and Proteins: Principles and Applications. ACS Symposium Series.* (Ed.: B. Ram Singh), ACS, Washington DC, **2000**, pp. 54-95.
[4] L. Nafie, *Vibrational optical activity: Principles and applications*, Wiley, Chichester, **2011**.
[5] a) J. Kessler, T. A. Keiderling, P. Bouř, *J. Phys. Chem. B* **2014**, *118*, 6937-6945; b) V. Parchaňský, J. Kapitán, J. Kaminský, J. Šebestík, P. Bouř, *J. Phys. Chem. Lett.* **2013**, *4*, 2763-2768; c) S. Yamamoto, X. Li, K. Ruud, P. Bouř, *J. Chem. Theory Comput.* **2012**, *8*, 977-985.

Research schedule

2017-2022

- Purification and isolation of representative ND samples.
- Surface modification of NDs.
- Large-scale preparation and isolation of the proteins and their isotope-labelled variants.
- Synthesis of model shorter peptides mimicking suggested binding domains.
- Acquiring spectral data on interaction of NDs with proteins.

2019-2022

- Ascertaining the structure of the ND surface.
- Multiple-spectroscopy strategies, analysis, theoretical binding model proposition.
- Suggestion of simple model peptide molecules.
- Synthesis of model peptides.
- Monitoring of the conformational behaviour during interaction with the surface.
  In vivo tests of novel compounds.

Publications and patents

Publications (Jimp)

	Jimp
2017	0
2018	1
2019	2
2020	3
2021	4
2022	5
Total	15

Nanoscale
Advanced Healthcare Materials
Nature Communications
Biomaterials
Small

Patents and patent applications

	Patents (granted)	International patent applications (filed)
2017	0	0
2018	0	0
2019	0	0
2020	0	1
2021	0	1
2022	1	0
Total	1	2

We expect IP protection in the following areas: Water-soluble nanoparticles with interesting FGF-modulating biological activity.

Cooperation with foreign institutions

- Prof. Francois Treussart from Laboratoire de Photonique Quantique et Moléculaire, Ecole Normale Superieure de Cachan and CNRS in Cachan, France
- Prof. Abraham Wolcott from the Department of Chemistry, San Jose State University in San Jose, California, USA
- Prof. Petr Král, University of Illinois at Chicago, USA.