Simulation Solutions for the Challenges in Nanomedicine

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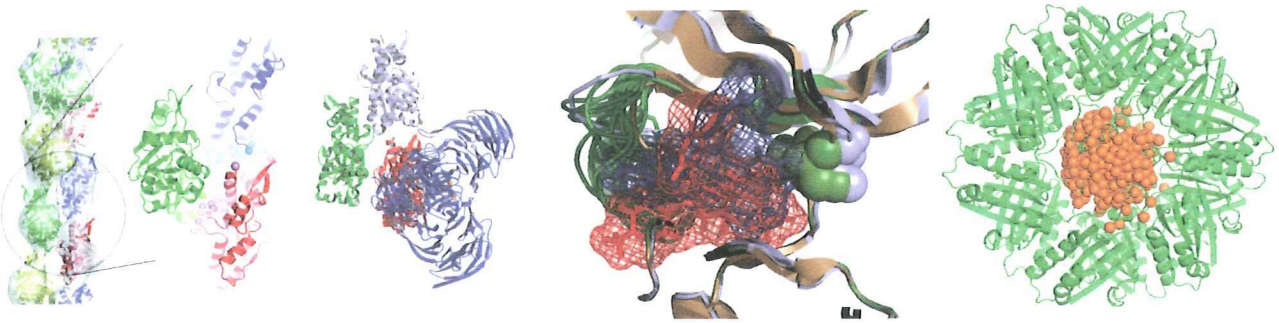
Driven by ever more powerful computational resources, simulation methods have become increasingly important to compliment experimental investigations in many scientific disciplines, including nanomedicine. In this talk I will review simulation methods to conquer this "time-scale gap", which have allowed significant progress towards understanding and rational design of biological function. Specifically I will report recent developments in drug discovery, including in-silico discovery of nanomolar compounds for blood coagulation; protein modeling and structure prediction, such as investigations of nanoparticle membrane permeability and Herg channel activity.



In-Silico Screeing: The medical and socioeconomic relevance of thromboembolic disorders promotes an ongoing effort to develop new anticoagulants. Heparin is widely used as activator of antithrombin, but incurs side effects. We screened a large database in-silico to find alternative molecules and predicted D-myo-inositol 3,4,5,6-tetrakisphosphate (TMI) to strongly interact with antithrombin. Isothermal titration calorimetry confirmed a TMI affinity of 45 nM, higher than the heparin affinity (273 nM). Functional studies, fluorescence analysis and citrullination experiments revealed that TMI induced a partial activation of antithrombin that facilitated the interaction with heparin and low affinity heparins. TMI improved antithrombin inhibitory function of plasma from homozygous patients with antithrombin deficiency with a heparin binding defect and also in a model with endothelial cells. Our in-silico screen identified a new, non-polysaccharide scaffold able to interact with the heparin binding domain of antithrombin. The functional consequences of this interaction were experimentally characterized and suggest potential anticoagulant therapeutic applications(2).

Design of Biocompatible Surfaces: Hydrophobins are fungal proteins with the ability to form immunologically inert membranes with a high stability and thus appear as attractive candidates for coatings of orthopaedic implants. However, cell adhesion on the surface of such implants is demanded for a better integration in the neighbouring tissue and hydrophobin surfaces do not mediate cell adhesion.

Here we investigated whether the class I hydrophobin DewA from Aspergillus nidulanscan be functionalised for a use on orthopaedic implant surfaces. DewA variants bearing one RGD sequence or the laminin globular domain LG3 binding motif were engineered. Surfaces of both variants allowed a significantly increased adhesion of mesenchymal stem cells (MSC), osteoblasts, fibroblasts and chondrocytes. The proliferation of MSC and their osteogenic, chondrogenic, and adipogenic differentiation potential were not modified on these surfaces. The insertion ofthe binding motifs RGD and LG3 in DewA did not increase the adhesion of Staphylococcus aureuson the hydrophobin surfaces. The engineered surfaces thus allowed an increased adhesion of MSC without interfering with their functionality and without leading to an increased risk for bacterial infections(3).

Protein Structure Predicition: Methods for protein structure prediction have matured in recent years and permit construction of models that can be validated subsequently with experimental methods. Here I will discuss a strudy on gas vesicle structure, which are proteinaceous structures produced by haloarchaea. The major structural protein GvpA forms a water excluding membrane with a hydrophobic inner and hydrophilic outer surface. We obtained the first structural model of the 76-amino acid protein GvpA by de novo modeling. Also, a dimer of GvpA was derived that allows explaining the formation of the GvpA monolayer in the gas vesicle wall. The hydrophobic inner surface is mainly constituted by anti-parallel

pinpointing contact sites between the dimers. GvpA mutants were tested for their ability to form gas vesicles in vivo. Mutations in □-helix I (R15A, R15K) and helix II (K60L). but also in the □ (I34M) affected the ability to form gas vesicles or resulted in an altered gas vesicle shape (E35A). In contrast. GvpA mutations S6A, S7A and F51Y had no effect on gas vesicle formation. The deletion of 5 or 7 amino acids at the C-terminus of GvpA had no effect, but a deletion of 11 amino acids resulted in the loss of function. All these mutants supported the structural features deduced from the model.

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