**Problem Set 1: Optical Resolution and Super-resolution Microscopy**

(a) **Diffraction Limit in Optical Microscopy**
The diffraction limit represents the fundamental limitation imposed on the resolving power of optical microscopes due to the wave nature of light. Explain the concept of the diffraction limit, including the underlying physical principles (such as the Airy pattern and Rayleigh criterion), and how it restricts the ability of conventional optical systems to distinguish two closely spaced objects in a microscopic image.

(b) **STimulated Emission Depletion (STED) Microscopy**
Super-resolution techniques have been developed to surpass the classical diffraction limit. Specifically, discuss STED microscopy by detailing its operating principle, including the roles of excitation and depletion lasers, and explain how controlled stimulated emission reduces the effective fluorescent spot size, thus enabling resolution beyond the diffraction limit.

(c) **Localization-based Super-resolution Microscopy (STORM/PALM)**
Describe the fundamental operation principle of localization-based super-resolution microscopy techniques, such as Stochastic Optical Reconstruction Microscopy (STORM) and Photoactivated Localization Microscopy (PALM). Explain how these methods achieve super-resolution by sequentially activating and precisely localizing individual fluorescent molecules, allowing reconstruction of high-resolution images from multiple sequential imaging cycles.

**Problem Set 2: DNA and Protein Sequencing Techniques**

(a) **Sanger Sequencing (Chain Termination Method)**
Explain the foundational principles behind Sanger sequencing, including the role of dideoxynucleotides (ddNTPs), and outline the main procedural steps involved in the method, such as DNA synthesis termination, separation of fragments, and sequence determination via electrophoresis.

(b) **Next-generation Sequencing (NGS)**
Describe the general principles of next-generation sequencing, emphasizing massively parallel sequencing approaches. Highlight the key technological advancements that contribute to the increased throughput, reduced sequencing costs, and improved accuracy compared to traditional methods such as Sanger sequencing.

(c) **Third-generation Sequencing Techniques: Zero-mode Waveguides and Nanopores**
Discuss the fundamental operating principles of third-generation sequencing technologies, specifically zero-mode waveguides (used in PacBio sequencing) and nanopore sequencing (such as Oxford Nanopore Technologies). Explain how each method detects nucleotide sequences in real-time, emphasizing their advantages such as longer read lengths, real-time analysis capabilities, and direct detection without amplification.

(d) **Protein Sequencing Using Nanopores**
Explain the theoretical basis and methodology for sequencing proteins using nanopore technologies. Discuss how nanopores detect and discriminate amino acids or peptides as they translocate through the pore, highlighting current challenges and future perspectives in nanopore-based protein sequencing.

**Problem Set 3: Nanoparticles and Biological Interactions**

(a) **Formation and Implications of the Protein Corona**
Define the protein corona phenomenon, explaining how it forms when nanoparticles interact with proteins in biological fluids such as blood or tissue fluids. Discuss the implications of protein corona formation on nanoparticle biodistribution, cellular uptake, targeting efficiency, and potential toxicity.

(b) **Challenges in Nanoparticle-based Drug Delivery Efficiency**
Discuss the key factors that contribute to the typically low efficiency (<1%) of nanoparticle-based drug delivery systems in reaching tumor sites. Include physiological barriers (such as the mononuclear phagocyte system, poor vascularization, and tumor microenvironment), nanoparticle design limitations, and systemic interactions.

(c) **Nanocarrier Design for Enhanced Tumor Drug Delivery**
Propose a comprehensive nanocarrier design tailored for effective delivery of anti-cancer drugs to tumor sites. Detail strategies to overcome challenges at the whole-body level (such as extended circulation time and immune evasion), organ/tumor-specific level (targeting through ligand-receptor interactions or tumor microenvironment responsiveness), and cellular level (enhanced cellular uptake and intracellular drug release).

(d) **Cancer Vaccines Using mRNA and Lipid Nanoparticles (LNPs)**
Describe the working principle behind cancer vaccines that utilize messenger RNA (mRNA) encapsulated within lipid nanoparticles. Explain how these vaccines trigger immune responses against cancer cells, focusing on the roles of mRNA encoding tumor-specific antigens, efficient intracellular delivery by lipid nanoparticles, antigen expression in antigen-presenting cells, and subsequent activation of the immune system to recognize and eliminate cancer cells.