1.3.28 Nanostructuring soft matter for targeted delivery at the cellular and sub-cellular scale

A significant body of knowledge was acquired at NEST on how soft-matter building blocks can be nanostructured in order to develop novel carriers tailored to therapeutic/diagnostic applications. Nanocarriers with a variety of synthetic identities and functions were developed and tested in in-vitro cellular models.

A variety of soft-matter building blocks were engineered to *either* target specific endocytic routes, *or* promote endosomal escaping *or* even avoid trapping and promote direct delivery of payloads into the cytoplasm. For instance, using small aminoacidic sequences (peptides) as platform, we demonstrated that vesicle-mediated transport (i.e. endocytosis) mediates their entry into cells at relatively low concentrations (i.e. nano- to micromolar), while direct translocation across the plasma membrane comes into play at higher concentrations (i.e. micro- to millimolar) [1]. In more detail, by a combination of UV-Vis analyses, NMR-based diffusion measurements and MD simulations it was demonstrated for the first time that the HIV-1 Tat arginine-rich peptide (Tat_{11}) is able to self-aggregate into dimers in a concentration-dependent manner (Fig. 1).

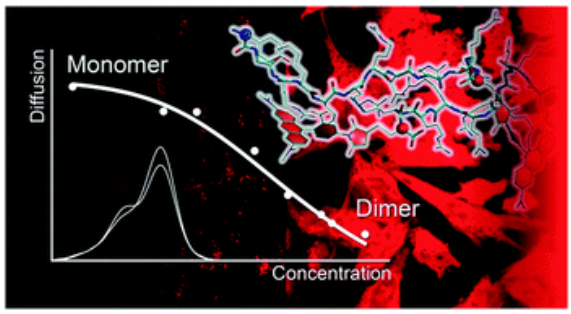

Figure 1. HIV-1 Tat arginine-rich peptide (Tat_{11}) is able to self-aggregate into dimers in both its fluorescently labeled and unlabeled variants (taken from Ref. [1])

The dimeric form is able to induce local perturbations of the plasma-membrane composition and/or integrity and favor peptide direct translocation. Experimental and *in-silico* data support the enhanced capability of peptide dimers in comparison to monomers to stabilize membrane pores [1, 2]. Worthy of mention, data also show how organic fluorophores (typically used for peptide labelling) have overall negligible kinetic and energetic effects on peptide transduction, yet they promote the process indirectly by favoring peptide aggregation. Similar conclusions were extended to either other cationic cell penetrating peptides (CPPs), such as Antennapedia (Ant) and nona-arginine (R9), and to amphipathic spontaneous membrane-translocating peptides [3].
To avoid trapping into endocytic vesicles at low peptide concentrations, it was demonstrated that the addition of the Tat-peptide module to well-established antimicrobial sequences (e.g. Cecropin-Melittin, or CM_{18}) is able to trigger a concentration-dependent detergent-like disruption of the vesicle membrane and massive release of the cargo into the cell cytoplasm [4]. The peptide concentration needed to produce an effect can be drastically reduced by the synergistic use of nanosecond electric pulses (NPs): by this approach, a nontoxic, high-yield, gene delivery system based on stable nanopore formation within vesicle membranes

followed by DNA release can be obtained, with no detectable perturbation of the plasma membrane [5].

There are selected cases in which the endocytic route cannot be avoided. For instance, it was demonstrated that the standard carriers used for lipid-mediated gene/drug delivery (i.e. liposomes) are invariably taken up by vesicle-mediated endocytosis, typically fluid-phase micropinocytosis, probably due to steric constrains [6]. By combining live-cell imaging, single-particle tracking microscopy, and quantitative transfection-efficiency assays, a mechanistic evaluation of the transfection barriers involved in lipid-mediated gene/drug delivery was achieved and the interplay between the nanostructure composition and its final performances unveiled. For instance, it was found that Lipofectamine, contrary to alternative formulations, is able to efficiently avoid active intracellular transport along microtubules, and the subsequent degradation of the payload within acidic/digestive lysosomal compartments, a process that can be eventually further tuned by modulating the cholesterol content of the liposomal carrier [7, 8]: this result is achieved by random Brownian motion of Lipofectamine-containing vesicles within the cytoplasm.

It was also demonstrated that, following exposure to biological milieus (e.g. after systemic administration), lipid-based carriers get spontaneously covered by an outer biomolecular corona (BC) that defines many of their biological outcomes, such as the elicited immune response, biodistribution, and targeting abilities. In this regard, by conducting confocal fluorescence microscopy experiments and image correlation analyses, it was shown that the BC can be modulated to promote a neat switch of the cell entry mechanism and subsequent intracellular trafficking, for instance from macropinocytosis to clathrin-dependent endocytosis, thus targeting a specific endocytic route [9] (Fig. 2).

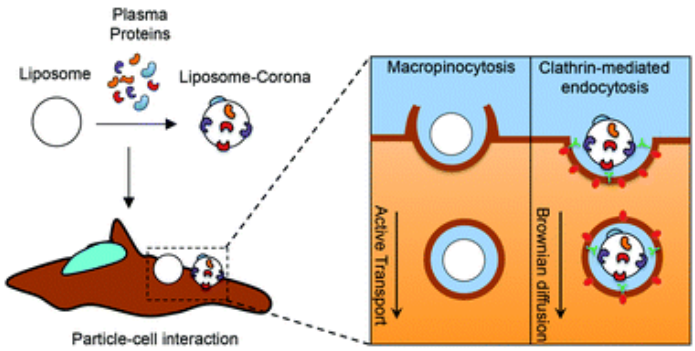

Figure 2. An apolipoprotein-enriched biomolecular corona switches the cellular uptake mechanism and trafficking pathway of lipid nanoparticles (taken from Ref. [9])

Depending on the specific *theranostic* application, the carrier can be engineered to include additional modules, as schematically sketched in the cartoon on the left. For instance, constant efforts at NEST are devoted to implement "Sensing" capabilities by means of GFP-derived mutants and/or environment-sensitive dyes; "Contrast Agents" are mainly built based on metallic and inorganic nanoparticles suitable for optical imaging and/or photoacoustic effects [10, 11]; finally, both existing and new "Drugs" are typically

employed in innovative configurations to increase their bioavailability and efficacy (e.g. doxorubicin linked to peptides or graphene-oxide [12]).

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