1. Record Nr. TD18044535

Autore	CARDOSO TRABUCO, MATILDE
Titolo	Innovative ferritin nanocages for drug-delivery and biotechnological applications [Tesi di dottorato]
Lingua di pubblicazione	Inglese
Formato	Tesi di dottorato
Livello bibliografico	Monografia
Note	diritti: info:eu-repo/semantics/openAccess In relazione con info:eu-repo/semantics/altIdentifier/hdl/11573/1114837
Sommario	The work presented in this thesis aimed to set out the basis for the rational design of innovative protein-based carriers for drug-delivery and biotechnological applications. In this context, ferritin stood out as promising protein system due to its remarkable characteristics. Ferritins are versatile biocompatible proteins scaffolds that display a cage-like structure that provides shielding of the cavity's content from harsh external conditions, and are amenable to modifications in a relatively straightforward manner. Previously published information noted that the proteic cage of ferritins presents an unexpected degree of plasticity in that it can undergo significant structural rearrangements and thus trap small molecules within the internal cavity, a process referred to as "encapsulation". However the assembled state of ferritin's cage has always been seen as an impermeable structure, in which the communication between the internal cavity and the exterior is uniquely governed by the gating effect of the channels located at the threefold symmetry axes. In order to clarify the process of molecule confinement within ferritin the internal cavity, a critical analysis of the process of ligand entry/release through the protein matrices of ferritins of different origin was carried. Thus the kinetics of the disulfide bond formation between DTNB and engineered cysteines at selected positions were

followed. Kinetics were found to be in the order of tens of seconds, a time frame likely to reflect the slow crossing of the protein matrix. The obtained data indicates that the protein matrix does not provide a significant barrier against bulky ligands such as DTNB, which, due to its dimensions (8-10 Å) and its net negative charge was thought unlinke to cross the protein shell. Within this vision, the technological effort of protein engineering for payload delivery may be most conveniently addressed to modification of the properties of the surface of the internal cavity rather than to possible rearrangements of the threefold channels. In an effort to develop versatile ferritin-based drug-delivery systems, archaeal ferritins appeared particularly promising as scaffolds. These proteins displays unique assembly properties and extreme thermodynamic stability, however lack the cell recognition properties of human ferritin. Thus a chimeric protein was designed in which the relevant recognition sequence of human ferritin was grafted into the corresponding sequence into archaeal ferritin surface exposed regions in order to confer specific recognition of human epitopes while keeping the unique salt-dependent assembly reaction. The construct structure was determined by X-ray crystallography and successfully shown to be actively uptaken via the Transferrin Receptor 1, a receptor known to be overexpressed in cancer cells. With the aim of improving current encapsulation methods the assembly properties of the novel archaeal-human chimeric ferritin nanocarrier were investigated and the effect of divalent cation investigated. Data demonstrated that physiological Mg2+ concentrations are sufficient to promote full assembly and that assembly takes place in a highly cooperative and fast manner, driven mostly by hydrophobic forces. However, at present, the effect of divalent cations has not been translated into a model of assembly mechanism, though, taken together, data indicates that subunit oligomerization may possibly follow an analogous mechanism as identified for cation-induced assembly of viral capsids. The understanding of the major forces governing the assembly provides key elements for the development of strategies for efficient encapsulation/ release of probes in a controlled way. On a different approach, the project aimed to exploit the versatility of ferritins for bioimaging applications by inserting an extra functional segment per monomer for binding of luminescent lanthanides, within mouse H ferritin’s cavity. This extra segment possesses one high affinity Terbium binding site provided by six coordinating aminoacid side chains and a tryptophan residue in its close proximity for FRET sensitization. Accordingly, the construct demonstrated lanthanide fluorescence detectable in the pM concentration range and demonstrated to be actively uptaken by selected tumor cell lines by confocal microscopy and FACS analysis of their FITC derivatives. Crystallographic data shown that introduction segment did not disrupt the cage assembly, and the presence of a total of 56 Tb(III) atoms per 24mer. These systems could be used for advanced cell imaging applications, merging the recognition capabilities of ferritins with the notable properties of lanthanide-based fluorescence.