Figure 1. Structure and architecture of T22-GFP-H6 building blocks (BB) and NPs. A. Modular organization of T22-GFP-H6 and the related constructs GFP-H6 and T22-H6. Relative sizes of boxes are only indicative. Precise details of T22-GFP-H6 NP construction can be found elsewhere [20]. The integrity and molecular masses of T22-GFP-H6, GFP-H6 and T22-GFP were confirmed by MALDI-TOF to be 30596.3, 27453.3 and 28359.6 respectively. B. Hydrodynamic size and pdi (polydispersion index) of the proteins indicated in panel A, determined by dynamic light scattering (DLS). Peak values refer to the average mode of the population, that always rendered a standard error lower than 0.00. C. Representative FESEM images of purified T22-GFP-H6 NPs, showing the cyclic organization of the oligomer. White bars indicate 20 nm. D. Surface representation of a T22-GFP-H6 NP from the major purification peak in the immobilized metal ion affinity chromatography (IMAC), in a top view (modelled with HADDOCK). Each T22-GFP-H6 protein monomer is differently coloured (modified from [20] and reproduced with permission of John Wiley and Sons). Size and organization of the model are compatible with the FESEM morphometry.

Figure 2. Cation-mediated NP disassembling and reassembling. A. Hydrodynamic size of T22-GFP-H6 NPs in absence (control, represented in green) or in presence of different molar ratios (1:1, in blue and 3:1, in red) of diverse free amino acids. Arginine (Arg) was tested to probe electrostatic interactions while tryptophan (Trp), histidine (His) and tyrosine (Tyr) to probe cation-pi contacts. Representative DLS plots are shown, indicating the value of the peak and pdi. B. EDTA-mediated disassembling of non-treated T22-GFP-H6 NPs, in green and +EDTA, in red. C. Cation-promoted reassembling of EDTA-treated protein T22-GFP-H6 NPs. GFP-H6 and non-treated T22-GFP-H6 NPs are included as references. Bars are clustered by the type of cations used in the reassembling process, that were added to a final molar ratio of 1:1 at exception of Ca^{2+} that was used at 1:2, the minimum concentration in which reassembling was observed. K^{+} was used at both the same concentration (black bar) and at the same ionic strength (blue bar) than metal ions to discard any unexpected influence or artefact. Protein complexes above the discontinuous line are considered as NPs (green) and below are considered BBs (red). Data expressed as $\overline{x}$ ± standard error of the mean (SEM), n= 3, and statistical comparisons are in relation to +EDTA group (ns: no significant, *p<0.05, **p<0.001).

Figure 3. Functional plasticity and manipulability of Ni^{2+} as an architectonic agent. A. Dose-dependent role of Ni^{2+} in the assembling and architecture of T22-GFP-H6 NPs, determined through the hydrodynamic size of EDTA-disassembled T22-GFP-H6 upon exposure to increasing amounts of Ni^{2+}. B. Hydrodynamic size variation of T22-GFP-H6 protein when: (a) incubated with EDTA at 1:1 molar ratio for 1 h; (b) dialyzed against sodium carbonate with salt buffer and treated with Wash buffer; (c) purified with IMAC

and eluted in elution buffer; (d) dialyzed against sodium carbonate with salt. The experiment was performed in consecutive steps. Data expressed as $\bar{x}$ ± SEM, n= 3, and statistical comparisons are *p<0.05, **p<0.001. We nomenclature is as in Figure 1.

Figure 4. Accommodation of Ni^{2+} in T22-GFP-H6 NPs. A. Immunodetection of IMAC-purified T22-GFP-H6 protein in non-denaturing gels (a), and of the same protein submitted to size-exclusion chromatography (SEC) to ensure the absence of BB and assembling intermediates that might occur in the pure protein samples (b). The bacteria soluble fraction was added to analyse the supramolecular conformation of T22-GFP-H6 protein (c). B. Hydrodynamic size of T22-GFP-H6 NPs disassembled with EDTA, and subsequently dialysed against the crude bacteria soluble fraction (BSF). As BSF was rich in bacterial proteins (BPs), sodium carbonate with salt buffer was also dialyzed against BSF as control. Nomenclature is as in Figure 1. Data expressed as $\bar{x}$ ± SEM, n= 3, and statistical comparisons are in relation to +EDTA group (*p<0.05). C. Direct Ni^{2+} chemical analysis by ICP-MS of T22-GFP-H6 NPs and EDTA-mediated disassembled T22-GFP-H6 proteins. In the inset, *in situ* visualization of a backscattered electron density, compatible with Ni^{2+}, in assembled T22-GFP-H6 NPs and EDTA-mediated disassembled T22-GFP-H6 proteins (morphometry and backscattered images). Sodium carbonate with salt buffer was also added as control (B). Data expressed as $\bar{x}$ ± SEM, n= 3, and statistical comparison is in relation to +EDTA group (*p<0.05).

Figure 5. A. Model of a T22-GFP-H6 NP in which all C-terminal histidine tag residues of each monomer are superimposed on the structure chosen as the His-Ni^{2+} (coloured atoms and red spheres) interaction model. The arrow indicates the GFP loop where the H6-tail segment was moved to in the construction T22-GFP-H6(Loop) (described in panel C). B. Detail of the superposition of His residues on the N-terminal tail of one of T22-GFP-H6 monomer of NPs. C. Schematic representation of T22-GFP-H6(Loop), in which the H6 segment is accommodated within the GFP, in the solvent-exposed loop indicated in panel A. The precise amino acid sequence of the protein is indicated, as well as the hydrodynamic size coincident with a disassembled form of the protein. The molecular mas of the protein was determined to be 30808.7 Da by MALDI-TOF.