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7-methylguanosine 5'-monophosphate analogues as molecular tools to investigate the role of human cytosolic 5' nucleotidase IIIB (cNIIIB)

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5'-nucleotidases are enzymes responsible for hydrolysis of nucleosides 5'-monophosphates to corresponding nucleosides and inorganic phosphate. 5'-nucleotidases are involved in the regulation of cellular levels of nucleoside 5'-monophosphates. Some 5'-nucleotidases are also involved in the deactivation of certain nucleoside-derived drugs [1]. A recently identified cytosolic 5'-nucleotidase IIIB (cNIIIB) shows preference towards 7-methylguanosine monophosphate (m^7GMP) as a substrate, which suggests its potential involvement in mRNA degradation [2]. However, biological function and structure of human cNIIIB are still unknown.

Here, we synthesized a series of m^7GMP analogues that could be used to modulate processes related to cNIIIB activity. Using high-throughput screening methods a library of mono- and diphosphate 7-methylguanine nucleotide analogues was tested as for inhibition of cNIIIB. Selected compounds were applied in cell extracts to investigate the role of human cytosolic 5'- nucleotidase IIIB in mRNA degradation pathways using a mass spectrometry-based assay. Based on the crystallographic structure of cNIIIB from *Drosophila melanogaster,* two fluorescent probes were designed and synthesized, which enabled us to develop a new method to study the binding preferences of cNIIIB.

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Nanoparticles as intermediaries in the interaction of proteins with x-ray

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Photodynamic Therapy (PDT) has gained relevance due to its applications in cancer treatments and has now stood out for its variety of applications, such as in the fight against neoplasic and inflammatory diseases, microbial infections and aging. The discovery and development of phototoxic proteins capable of producing reactive oxygen species aligned with the development of Optogenetics makes it possible to use genetically encoded photosensitizers (PSs), increasing the specificity of the treatment. However, when it comes to *in vivo* use, optical techniques are limited by the low penetration of UV-visible light into biological tissues. To overcome this limitation, the use of X-rays has been suggested as energy source of excitation of PSs, due to their high penetrability in soft tissues. Giving that most photosensitizers (PSs) have absorption coefficients that are comparatively high at visible wavelength, the X-ray-induced sensitizer (XS) usually comprises traditional PSs and scintillation nanoparticles (ScNP). Considering for the first time the use of the proteins GFP, KillerOrange and KillerRed with the scintillant nanoparticle LaF3.Tb as XS, this work presents a characterization of the system protein-ScNP and its potential use in PDT. To this end, the structure, stability and quantum yield of fluorescence and generation of reactive oxygen species were evaluated upon X-ray stimulation of the system protein-ScNP. We also tried to shed light on the mechanisms of interaction between proteins and nanoparticles, seeking for strategies to increase the transfer of energy from ionizing radiation to biomolecules in order to improve the efficiency of the techniques mentioned above.

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IMPROVING LOOP MODELING PREDICTION

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Prediction of protein loop structures is crucial for protein structure modeling, structural refinement, antibody design, or ion channels modeling. Here we present an improved version of our loop modeling online service: Random Coordinate Descent (RCD+ http://rcd.chaconlab.org/). This server combines an *ab initio* loop closure algorithm with a full-atom refinement in Rosetta. Now it includes a novel knowledge-based pairwise potential (KORP) which takes into account information of the relative position and orientation per residue. KORP and an extensive parameter optimization significantly improve the prediction accuracy of the server. Moreover, superior efficiency has been achieved by drastically reducing the number of loop candidates to be further refined, in particular in the more challenging longer loop cases (>10 residues). The approach has been successfully validated with several standard loop benchmarks. Interestingly, promising results were obtained even in one of the most challenging applications scenarios: the H3 loops prediction of the antibody complementary determining regions.

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Evaluation of PAPS analogs as inhibitors for sulfotransferases using an MST-based assay.

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Amyloids have been exploited to build up amazing bioactive materials. In most cases, short synthetic peptides constitute the functional components of such materials. The controlled assembly of globular proteins into active amyloid nanofibrils is still challenging, because the formation of amyloids implies a conformational conversion towards a β-sheet-rich structure, with a concomitant loss of the native fold and the inactivation of the protein. There is, however, a remarkable exception to this rule: the yeast prions. They are singular proteins able to switch between a soluble and an amyloid state. In both states, the structure of their globular domains remains essentially intact. The transit between these two conformations is encoded in prion domains (PrDs): long and disordered sequences to which the active globular domains are appended. PrDs are much larger than typical self-assembling peptides. This seriously limits their use for nanotechnological applications. We have recently shown that these domains contain soft amyloid cores (SACs) that suffice to nucleate their self-assembly reaction. Here we genetically fused a model SAC with different globular proteins. We demonstrate that this very short sequence act as minimalist PrDs, driving the selective and slow assembly of the initially soluble fusions into amyloid fibrils in which the globular proteins keep their native structure and display high activity. Overall, we provide here a novel, modular and straightforward strategy to build up active protein-based nanomaterials at a preparative scale.

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Amyloid proteins as synthetic devices for biotechnological purposes

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RepA-WH1 is a synthetic prion-protein that causes an amyloid proteinopathy in bacteria (1, 2). Synthetic biology seeks to implement functions of biotechnological interest through the design of minimal artificial systems. Using RepA-WH1 as a model for amyloid formation, our goal is to develop new biotools based on the properties of amyloids.

In this poster, we present results in three main aspects of research in our laboratory:

1) We have explored the conformational dynamics of RepA-WH1 through NMR $\{^1H\}$-^{15}N relaxation and H/D exchange kinetics measurements, including titration experiments with an inhibitor of amyloid formation (S4-indigo). Our objective is to define the initial stages of the conformational change that drives amyloidogenesis and the relevant interactions for amyloid remodeling.

2) We have recently achieved control of RepA-WH1 amyloidogenesis through optogenetics. For this purpose, the N-terminus of WH1-mCherry was fused to a blue light-responsive plant domain (LOV2). The expression of these chimeras under blue light illumination leads to the assembly of oligomers that are cytotoxic in *E. coli*, while in darkness large intracellular amyloid inclusion are formed which are compatible with bacterial proliferation (3). This tool provides direct control of amyloidogenesis with light.

3) Finally, we are working with cytomimetic lipid containers (GUVs) for exploring the *in vitro* formation of amyloid RepA-WH1 aggregates with a minimal set of components. We have achieved the solubilization of these aggregates by chaperones (Hsp70 + Hsp40 + NEF) in such cytomimetic containers (4, 5). This system is now being adapted for its usage with the optogenetic devices.

Together these studies constitute useful devices to explore general routes of toxicity of amyloids and to develop new tools amenable for environmental and biomedical applications.

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