MS12-P3 Structural and dynamics studies of human phenylalanine hydroxylase, a highly regulated allosteric enzyme

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Phenylalanine hydroxylase (PAH) is a tetrahydrobiopterin (BH_4)-dependent enzyme that catalyses the rate-limiting step in the degradation of phenylalanine (L-Phe). Excessive amounts of L-Phe is toxic to the brain and in patients with the disease phenylketonuria (PKU), a dysfunctional PAH thus leads to irreversible brain damage if the patient does not follow a life-long diet with restricted protein content. Understanding the conformation and dynamics of the enzyme, as well as the misfolding and instability changes caused by the more than 800 PKU-associated loss-of function mutations, is an essential requirement in the search of therapeutic strategies towards PKU, such as pharmacological chaperones. Mammalian PAH is a large tetrameric (200 kDa) enzyme and each of the identical subunits consists of three domains: an N-terminal regulatory domain (RD), a central catalytic domain (CD) and a C-terminal oligomerization domain (OD). In the inactive state, the long, unstructured N-terminus of the RDs covers the active site entrances. However, upon L-Phe binding, the flexible hinges between the domains promote a large conformational change resulting in displacement of the N-terminus, a cooperative increase in activity and stabilization of the high-activity state. We are at present investigating the conformation and dynamics of human PAH using structural techniques such as X-ray crystallography and SAXS, in combination with binding studies and molecular dynamics simulations. We are interested in unveiling the catalytic mechanism of human PAH as well as the regulatory conformational events elicited by its substrate and cofactor BH_4. Also, we are investigating how the structure and dynamics are affected by recurrent PKU mutations as a base in the design of novel therapies for PKU. Our recent results on this matter will be presented.

Keywords: X-ray crystallography, SAXS, molecular dynamics simulations, phenylketonuria

MS12-P4 DNA I-motifs: Beyond the Double Helix

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I-motifs are four-stranded DNA structures made up of cytosine-rich sequences.[1] These structures are held together by hemi-protonated cytosine$^+$- cytosine base pairs (Fig. 1A) to form an intercalated motif (hence the name i-motif), therefore, proving to be more stable in acidic conditions. This unique property helped produce the first DNA molecular motor to be driven by pH changes. Cytosine-rich sequences have also been detected in promoter regions of several oncogenes, making i-motifs an attractive subject for gene transcription modulation along with DNA nanotechnology.

The i-motif can form as either an inter- or an intramolecular structure (Fig. 1B). However, only six i-motif crystal structures have been reported to date; all of which are tetramolecular, even though i-motifs *in vivo* would exist as intramolecular. UV and synchrotron radiation CD (srCD; beamline B23 at Diamond Light Source) spectroscopy were used to study the structural stability of intramolecular i-motifs. Our results showed that i-motifs with shorter loop lengths exhibit the highest stability.[2] Crystallisation trials based on these initial results will be reported along with previously recorded i-motif crystals grown in new conditions. I will also be reporting the diffraction of $d(CCCT)_4$ crystals (Fig. 1C) at 0.68 Å at beamline I02, illustrating the advances in modern-day DNA crystallography via synchrotron radiation. Combination of results from the mentioned instrumental approaches shows that these methods are actually complementary.

References

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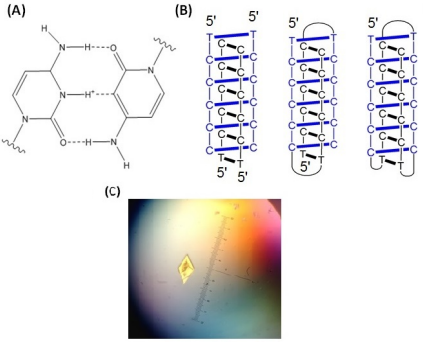

Figure 1. (A) Cytosine$^+$- cytosine base pairing in i-motifs. (B) Schematic diagrams of tetramolecular (left) bimolecular (middle) and intramolecular (right) i-motifs. (C) 30×25×15 micron crystal of d(CCCT)$_4$.

Keywords: i-motif, DNA, biophysical

MS12-P5 Crystallography Platform at the Pasteur Institute

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The goal of the crystallography platform is to provide research teams working in the field of macromolecular crystallography at Institut Pasteur with the expertise and technology for high throughput crystallization, X-ray diffraction measurements, and crystallographic computing as a core facility. Our second mission is to offer expertise in bio-crystallography, from crystallization of selected targets to resolution of 3D crystal structures by participating as a partner in research projects involving structural studies of single proteins and protein complexes. These projects arise from direct collaboration with research groups at Institut Pasteur and outside organisations. Depending on the expertise of the users, three options can be offered: service provision, instrument allocation, and scientific collaboration. Service provision, which corresponds to the automated crystallization experiments performed in standard conditions, is the option used by the crystallographers. If initial crystallization trials are successful, the platform assists users to reproduce and optimize the crystallization conditions in order to obtain suitable crystals for X-ray data collection. Since 2010, the platform has been involved in more than 24 scientific collaborations in association with 14 research units from 8 scientific departments of the Institut Pasteur and 6 laboratories from other institutions (French or foreign), leading to our co-authorship of 32 peer-reviewed publications. These projects cover many disciplines related to infectious diseases and human health, including defense mechanisms against pathogens, antibiotic resistance, regulation pathways, genetic disorders and drug design.

In our poster, we present the instrumentation and robotics available in the platform and a summary of results obtained during the last five years.

Keywords: High throughput, crystallization, pipeline