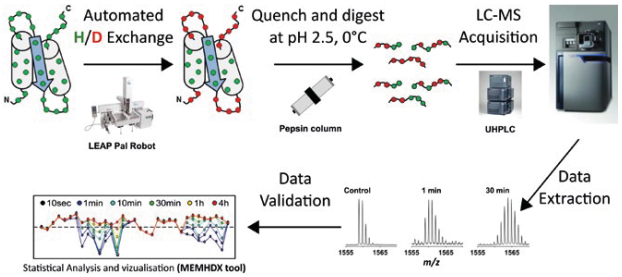
MECHANISM OF ACTIVATION OF A MAJOR TOXIN INVOLVED IN WHOOPING COUGH

The CyaA protein is a toxin produced by *Bordetella pertussis*, the bacteria responsible for whooping cough. When it intoxicates target cells, CyaA binds to a molecule known as calmodulin, an interaction that leads to CyaA activation. Scientists from the Institut Pasteur and the CNRS coupled SAXS and SR-CD studies performed at SOLEIL with hydrogen-deuterium exchange monitored by mass spectrometry (HDX-MS) in an integrative structural biology approach. The combined results allowed them to elucidate the relationship between the structure and activation of CyaA. The mechanism involves calmodulin binding to an unstructured region of CyaA that folds upon binding. The resulting structural rearrangement of the catalytic domain of CyaA leads to its activation.

Adenylate cyclase toxin (CyaA) is a major virulence factor of *Bordetella pertussis*, the causative agent of whooping cough [1]. This disease, which can affect people of all ages, is particularly severe and potentially fatal for infants and vulnerable individuals. The CyaA toxin contributes to the early stages of bacterial colonization of the lungs in people infected by *B. pertussis*. CyaA is synthesized and secreted by the bacterium in an inactive form. The CyaA toxin employs a unique mechanism to access the cytosol in our cells (the liquid phase inside cells): the catalytic domain is directly transported via the plasma membrane of target cells through a process known as membrane translocation.

HOW DOES CALMODULIN ACTIVATE CyaA?

Calmodulin is a highly conserved eukaryotic protein that interacts with a wide variety of other proteins. It controls their activities in response to variations in concentrations of intracellular calcium. After invading eukaryotic target cells, the catalytic domain of CyaA is activated by calmodulin to synthesize huge quantities of cAMP. cAMP is a molecule used in low concentrations as an intracellular messenger. But the large quantities produced by CyaA impair cell physiology and ultimately lead to cell death. The activation of CyaA by calmodulin – an act of molecular piracy – has been known for some time, but the molecular activation mechanism remained a mystery. Researchers from the Institut Pasteur and the I2BC used an integrative structural biology approach combining several biophysical techniques to characterize the structural changes in the CyaA catalytic domain and in calmodulin when they interact: size exclusion chromatography small-angle X-ray scattering (SEC-SAXS) data [2] were recorded on SWING, synchrotron radiation circular dichroism (SR-CD) spectra were obtained using DISCO while hydrogen/deuterium exchange monitoring by mass spectrometry (HDX-MS) was performed at the Institut Pasteur (Fig. 1) [3].



1 - Overview of a typical HDX-MS workflow. The protein under investigation is equilibrated in the presence and absence of its partner (protein or ligand). The mixture is continuously labelled in excess deuterium at room temperature and physiological pH and sampled over several time points. The labelling reaction is quenched and label preserved by rapidly reducing the pH to 2.5 and the temperature to 0°C. Protease digestion generates peptide fragments which can be used to localize the exchange behaviour to specific regions within a protein. Peptides are injected into a cooled nanoACQUITY HDX UHPLC™ system connected to a Synapt™ G2-Si HDMS™ mass spectrometer (Waters). Mass spectrometric data is processed, extracted, analysed and visualized using the MEMHDX software (3). Triplicate measurements are routinely obtained for each labelling experiment.

TRANSITION FROM STRUCTURAL DISORDER TO ORDER IN CyaA

A region of 75 amino acids in the catalytic domain of CyaA was shown to be disordered in solution and to act as a bait to capture calmodulin (Fig. 2, left). Binding induces significant folding in this region, a prerequisite for CyaA activation (Fig. 2, right). Beyond the region where interaction occurs between calmodulin and the catalytic domain, the formation of the complex also triggers allosteric changes and stabilizes the distant catalytic site. It is interesting to note that a catalytic loop (the red star in Fig. 2, right) is maintained in a highly flexible state, which is essential for effective enzyme catalysis (more than 1,000 reactions per second!), allowing rapid association/dissociation of substrates (ATP) and products (cAMP and pyrophosphate). The production of cAMP irreversibly impairs the physiology of the immune system cells in the lungs, giving B. pertussis bacteria free rein to colonize this region.

MAKING USE OF STRUCTURAL DISORDER TO DEFEND AGAINST THE TOXIN

This research opens up new avenues for the identification of CyaA inhibitors. The aim is to identify molecules capable of binding to the flexible region in the catalytic domain instead of calmodulin, yet without inducing the allosteric effects that structure and activate the catalytic site.

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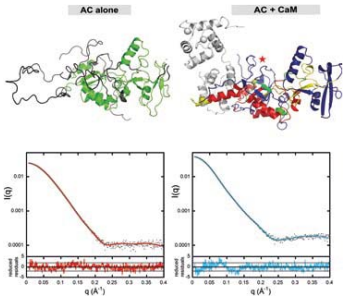

2 - Structural models of the catalytic domain of CyaA isolated and activated by calmodulin. **Left:** structural model of the catalytic domain (AC) of isolated, inactive CyaA and below the resulting adjustment of its calculated scattering pattern (red curve) against experimental data (black dots). The regions folded into helices and sheets are represented in green, while the regions characterized by structural disorder are represented as a black line. Structural disorder prevents catalytic activity. **Right:** the active enzyme complex, formed by the catalytic domain of CyaA (in colour) and calmodulin (in grey) and below the resulting adjustment of its calculated scattering pattern (blue curve) against experimental data (black dots). The stabilization of the catalytic domain induced by calmodulin binding is represented by the colour gradient red, yellow, green and blue; the red and yellow regions correspond to the maximum stabilization (observed with HDX-MS). The flexible catalytic loop is indicated by a red star. The structural models were obtained using experimental SAXS data recorded on SWING while incorporating results from HDX-MS and SR-CD experiments.

DISCO & SWING BEAMLINES

ASSOCIATED PUBLICATION

Calmodulin fishing with a structurally disordered bait triggers CyaA catalysis, PLOS Biology, December 29, 2017
D.P. O'Brien, D. Durand, A. Voegele, V. Hourdel, M. Davi, J. Chamot-Rooke, P. Vachette, S. Brier, D. Ladant & A. Chenal.

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