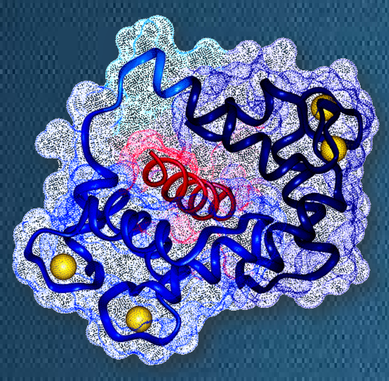

Rediscover and actualitzation of the sequence motif for calmodulin recognition

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Introduction

Calmodulin (**CaM**, an abbreviation for **CAL**cium-**MODUL**ated prote**IN**) is a well characterized protein which has been the objective of a lot of studies because it has a biologic important role in the cells. Moreover, the characterization of its sequence motif has not been really studied. Sequence motif for CaM recognition was studied in the 80s and the 90s, culminating in an article from 1997 wrote by F. Friedberg and A. R. Rhoads (1). In this article the authors summed up all the sequence motif discovered until their days in one classification. In the years 2001 , 2002 and 2003 some investigators improve and actualize the knowledge about it. That is the information we can observe on table 1 (yellow part).

The genome discovery along with technologic improvements have given us the opportunity to know lot of calmodulin target proteins. Some of these proteins can not be classified in any of the motif represented in the table 1, and that is why some people, including me, think that we do not really know the sequence motif for CaM recognition and there are more motif to find. This will be the aim of my project. For example, some people had evidence of the 1-12 motif, but it has not been accepted yet (2).

In conclusion, the hypothesis and objectives of my project are to discover a new sequence motif of CaM recognition (such possible 1-18 and 1-20 motif) and try to actualize the actual known motif. I will also determine the affinity of the interaction and I classify it (3).

Motif class	Subclasses	Sequence motif
1-10		
	1-5-10	xxx(FILVW)xxxx(FAILVW)xxxx(FILVW)
	Basic 1-5-10	(RK)(RK)(RK)(FAILVW)xxxx(FILV)xxxx(FILVW)
	1-10	(FILVW)xxxxxxxx(FILVW)
1-14		
	1-5-8-14	(FILVW)xxx(FAILVW)xx(FAILVW)xxxxx(FILVW)
	Basic 1-8-14	(RK)(RK)(RK)(FILVW)xxxxxx(FAILVW)xxxxx(FILVW)
	1-8-14	(FILVW)xxxxxx(FAILVW)xxxxx(FILVW)
	1-14	(FILVW)xxxxxxxxxxxx(FILVW)
1-16		
	1-16	(FILVW)xxxxxxxxxxxxx(FILVW)
IQ		
	IQ	(FILV)Qxxx(RK)Gxxx(RK)xx(FILVWY)
	IQ-like	(FILV)Qxxx(RK)xxxxxxxx
Others		
	1-12	KKHLQRPIFRLRCLVKQL EKMWQRLKGILRCLVKQL KKMWIKLRSLLRYMVKQL KATFRAITSTLASSFKRR KFKVICLTVLASVRIYYQ RRLIDAYAFRIYGHWVKK RSRWWRIRSIFALVFRRR
	1-18??	XXXXXXXXXXXXXXXXXXXXXX
	1-20??	XXXXXXXXXXXXXXXXXXXXXX

Table 1. The different sequence motif of CaM recognition accepted in yellow. In orange there is the 1-12 which has not been accepted yet and other possible motif not discovered.

Methods

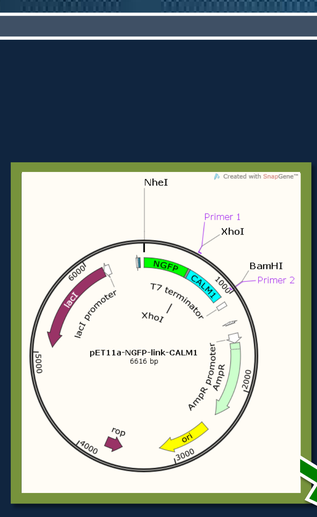

Fig. 1. Plasmid pET11a-NGFP-link-CALM1 which carries the amino acids 1 to 157 of the N.-terminal of the GFP, bonded using a linker to calmodulin (gene CALM1)

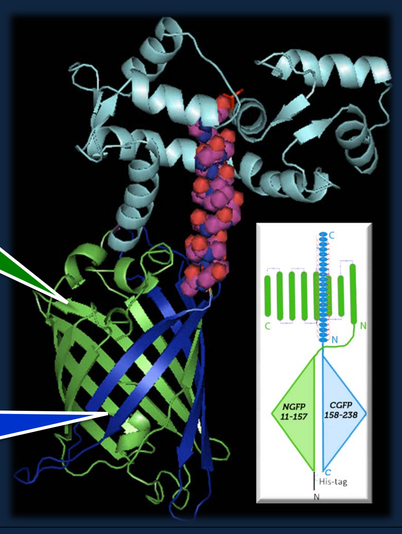

Fig. 2. A cartoon model of the ribbon structure of split GFP fused with the calmodulin (blue) and the peptide (purple). The model has been produced using PyMOL and the pdb files 1ema (5), 3cln and 1xnl chopped.
In the white square we can see the topology of GFP fragments fused to the CaM protein and the peptide.

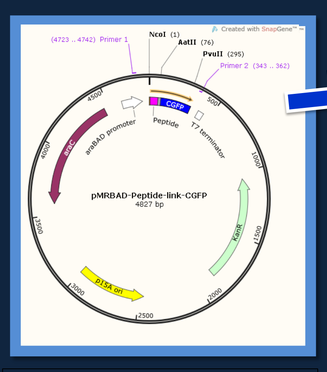

Fig. 3. Plasmid pMRBAD-peptide-link-CGFP carrying the designed peptide, a linker sequence and the C-terminal fragment of the GFP (amino acids 158 to 238).

BiFC

The major method I will use to realize this project is the Bimolecular fluorescence complementation (BiFC) because this technique is perfect to detect the interaction between CaM and the target peptide.

We will perform BiFC by transforming the cells with two plasmids that will carry both fragments of GFP (one fragment per plasmid) and the calmodulin or a designed peptide. The first plasmid called pET11a-NGFP-link-CALM1 (Fig. 1)(4) will consist in the N-terminal fragment of the GFP protein (amino acids 1 to 157) bond to a linker to the CDS of the gen CALM1, the calmodulin protein. The second plasmid called pMRBAD-peptide-link-CGFP (Fig. 3)(4) will consist in the C-terminal fragment of the GFP (amino acids 158 to 238) bond to a peptide designed randomly in some positions.

These two plasmids will be introduce to the cells. If there is an interaction between CaM and the peptide, both fragments of the GFP will reassembly (Fig. 2) becoming active. This activation of the GFP will cause and emission of green fluorescence that we can detect.
Observing the brightness that the interaction between the different peptides and the CaM produce, we will classify the affinity/stability of the interaction (3), creating a new way to characterize the interactions.

Sequencing and peptide design

Finally, we will also use the DNA sequencing because this will be the way to determine with which peptides has CaM interacted, and the way to obtain the sequence that we will try to classify. With these methods we can obtain a lot of sequences to analyse that interact with CaM.

The peptide production will be ordered to an specialized company. Tacking the consensus sequence (KRR)IPSWTTVILVKSMLRKRSFG(SSM) we will create some random positions that can be interesting for the project. These positions are shown below:

KRR **I** P S W **T** T V **I** L **V** K **S** M **L** R **K** R **S** F **G** SSM
1 5 8 10 12 14 16 18 20

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