SCUOLA DI DOTTORATO DI RICERCA IN SCIENZE BIOCHIMICHE, NUTRIZIONALI E METABOLICHE

DOTTORATO DI RICERCA IN BIOCHIMICA

XXII CICLO

Role of the Casein phosphopeptides and Vitamin D on calcium uptake and cell functions in human cancer intestinal cell lines differentiated in culture: a possible correlation between nutrients and colon cancer

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CASEIN PHOSPHOPEPTIDES

α_{s1}-Casein

45 63
-Gly-Ser-Glu-Ser-Thr…Glu-Ser-Ile-Ser-Ser-Ser-Glu-Glu-
P P P P P P

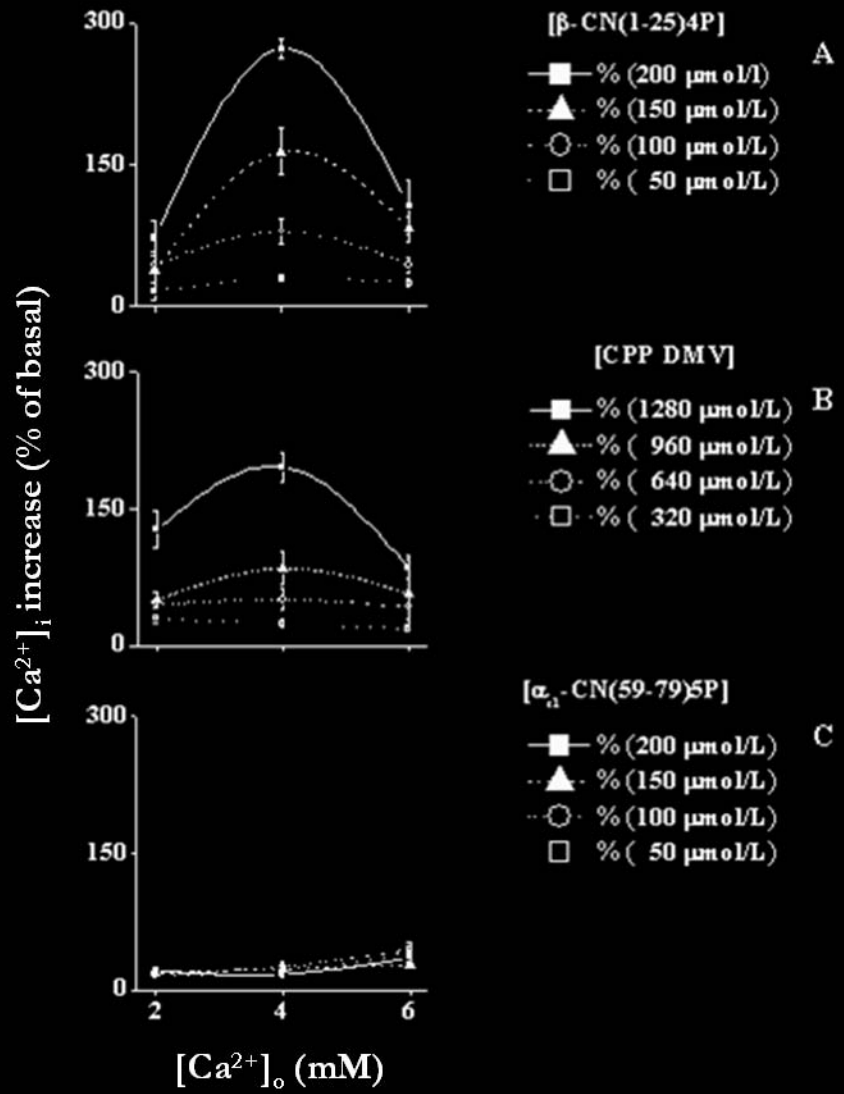
α_{s2}-Casein

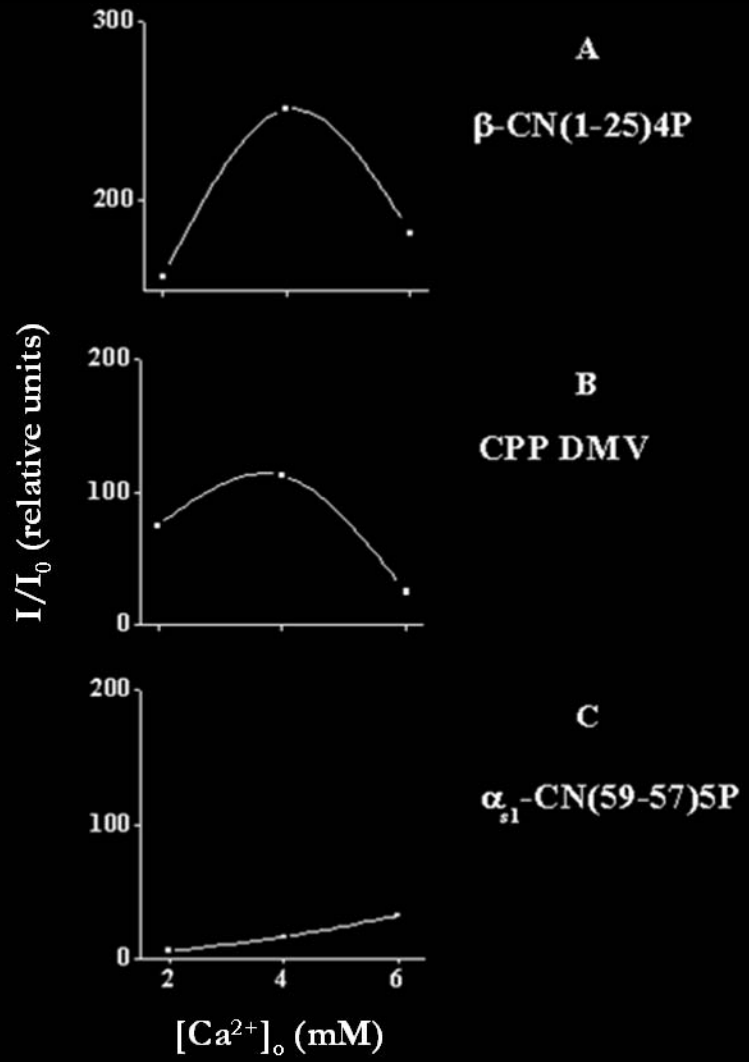
55
-Gly-Ser-Ser-Ser-Glu-Glu-Ser-Ala-Glu-Val-Ala-Thr-Glu-Glu-Val-Lys-
P P P P

β-Casein

13 34
-Val-Glu-Ser-Leu-Ser-Ser-Ser-Glu-Glu…Gln-Ser-Glu-
P P P P P

BIOLOGICAL ACTIVITY OF CPP





Gravaghi et al. *FEBS J.* 2007, 274(19):4999-5011

BIOLOGICAL ACTIVITY OF CPP

Macromolecular complexes CPP-Ca^{2+} represent the bioactive form of casein phosphopeptides

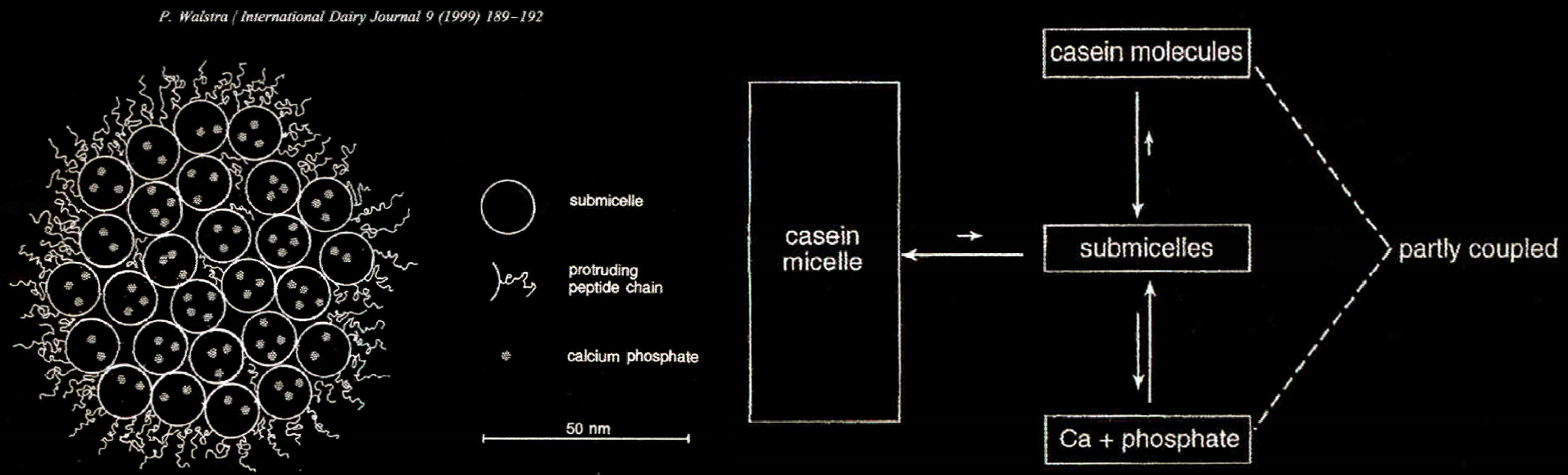
Gravaghi et al. *FEBS J.* 2007, 274(19):4999-5011

CALCIUM TITRATION IN CPP-DMV SOLUTIONS

$CaCl_2$ added in KRH buffer (mM)	CPP-DMV Ca_{free}/Ca_{bound}
0	0 / 0
2	0.19 / 1.81
4	1.26 / 2.74
6	2.75 / 3.25

AIM OF THE STUDY

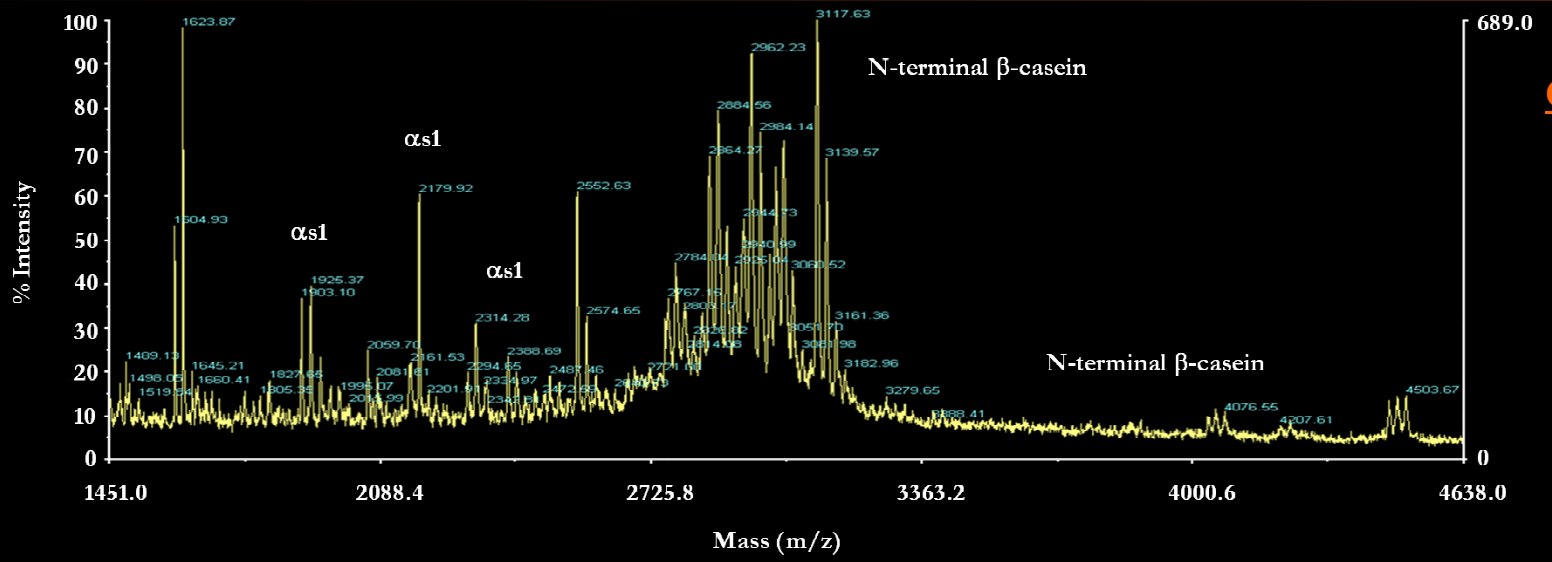
Which fraction of the calcium (free calcium or CPP-bound calcium) is involved in the calcium uptake by the cells?



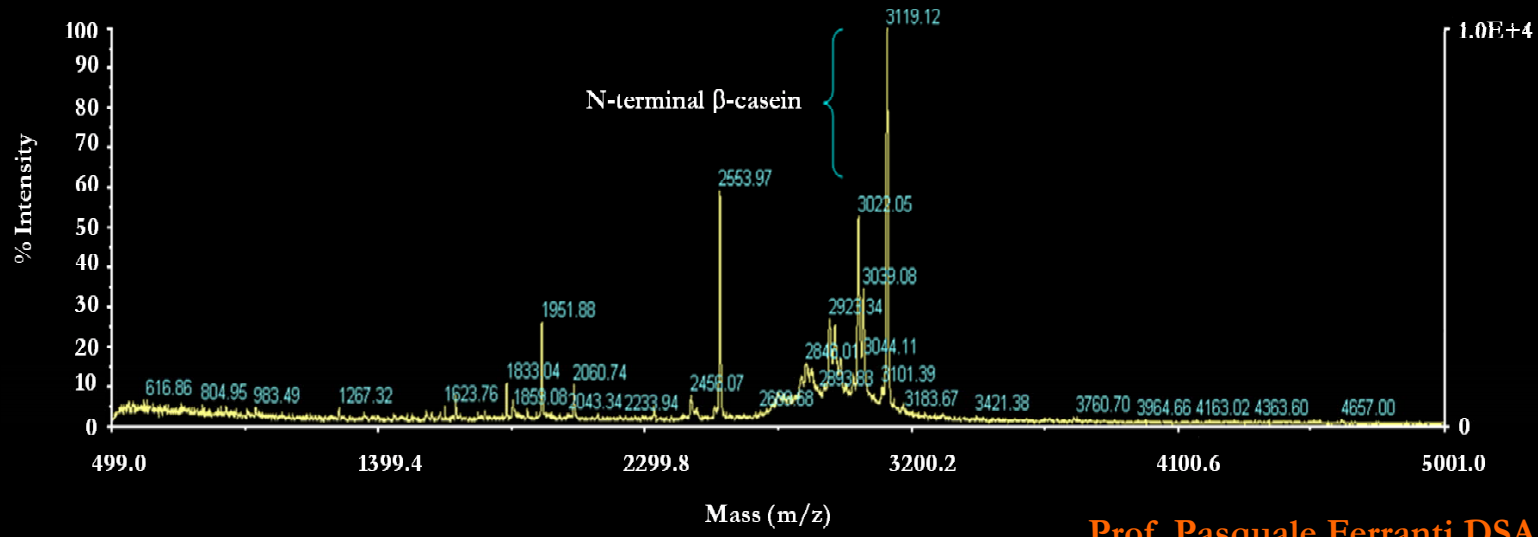
CPP-DMV vs CPP-MD

	CPP-DMV (CE 90 CP III)	CPP-MD (PEPTIGEN 110)
CPP content	90.5%	95%
Ca^{2+} content	0%	6.6%
P content	3.7%	3.2%
N/P ratio	3.7mol/mol	7.8mol/mol
Ser/P ratio	0.85mol/mol	0.97mol/mol

MALDI-TOF-MS ANALYSIS OF CPP-DMV AND CPP-MD



CPP DMV



CPP MD

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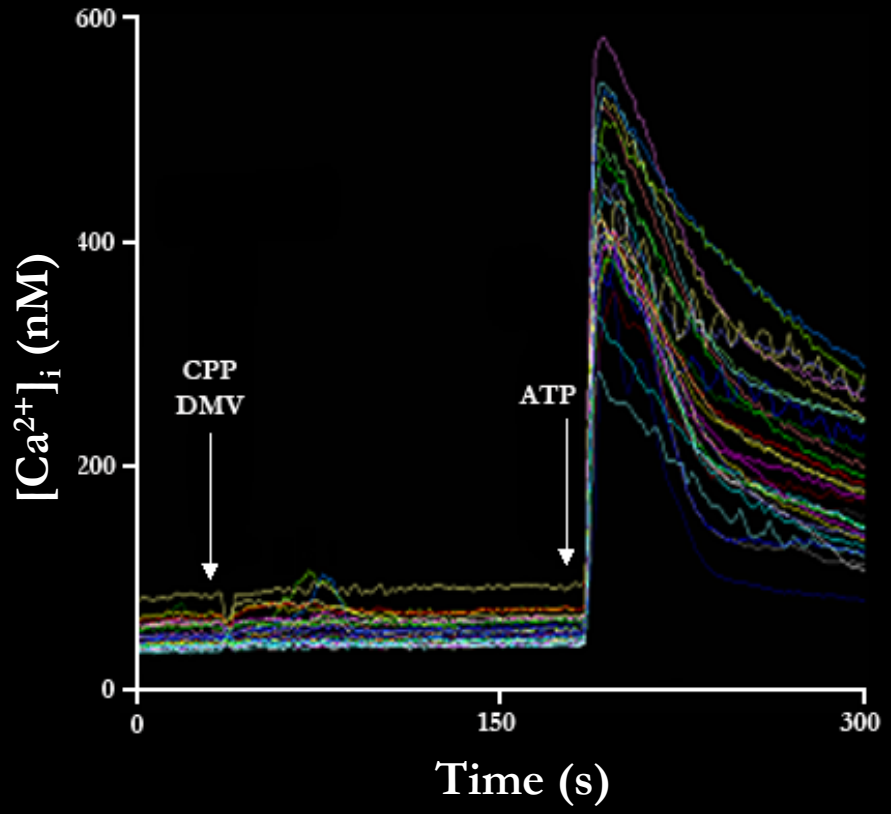
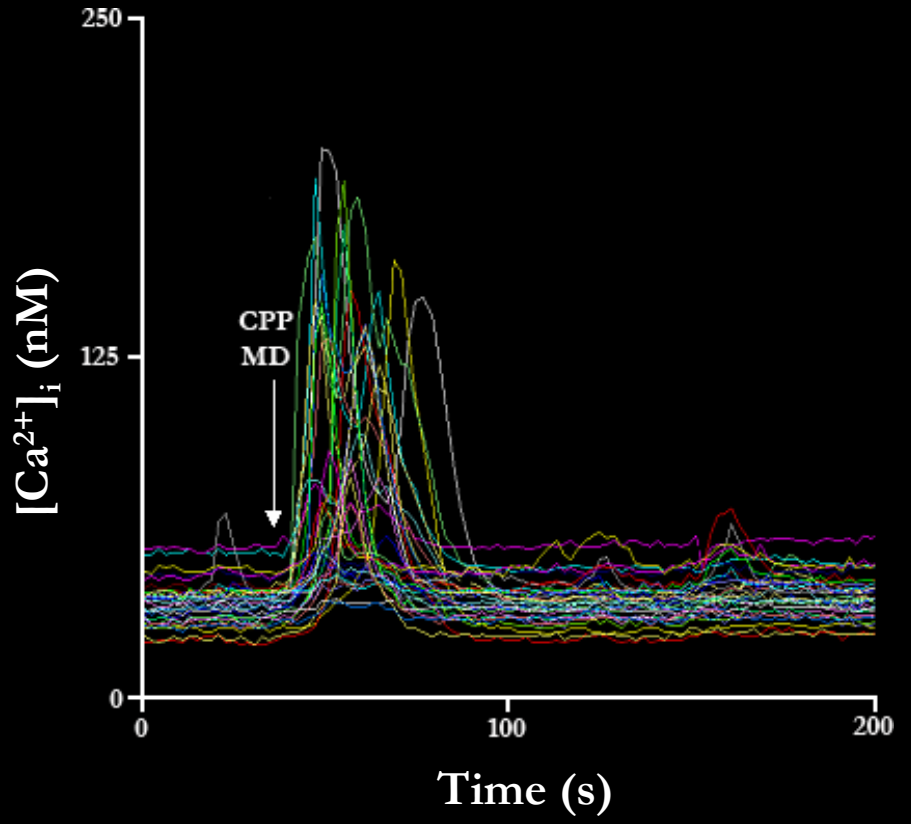
EFFECT OF CPP ADMINISTRATION ON $[Ca^{2+}]_i$





$[Ca^{2+}]_o$= 0mM

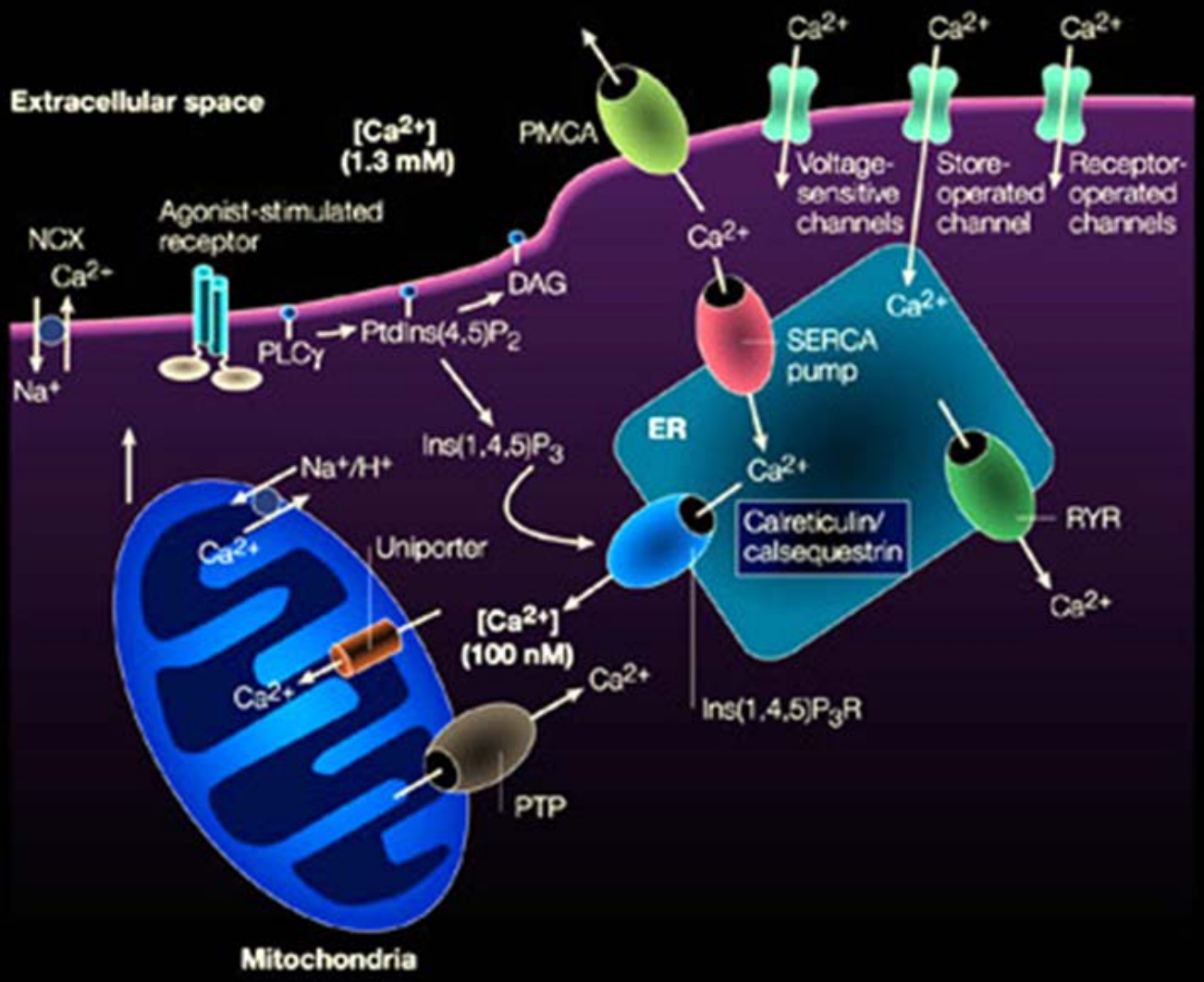
POTENTIAL MECHANISMS RESPONSIBLE FOR CPP-INCREASED $[Ca^{2+}]_i$

- Interaction of CPP with a membrane component stimulating the Ca^{2+} release from the intracellular stores

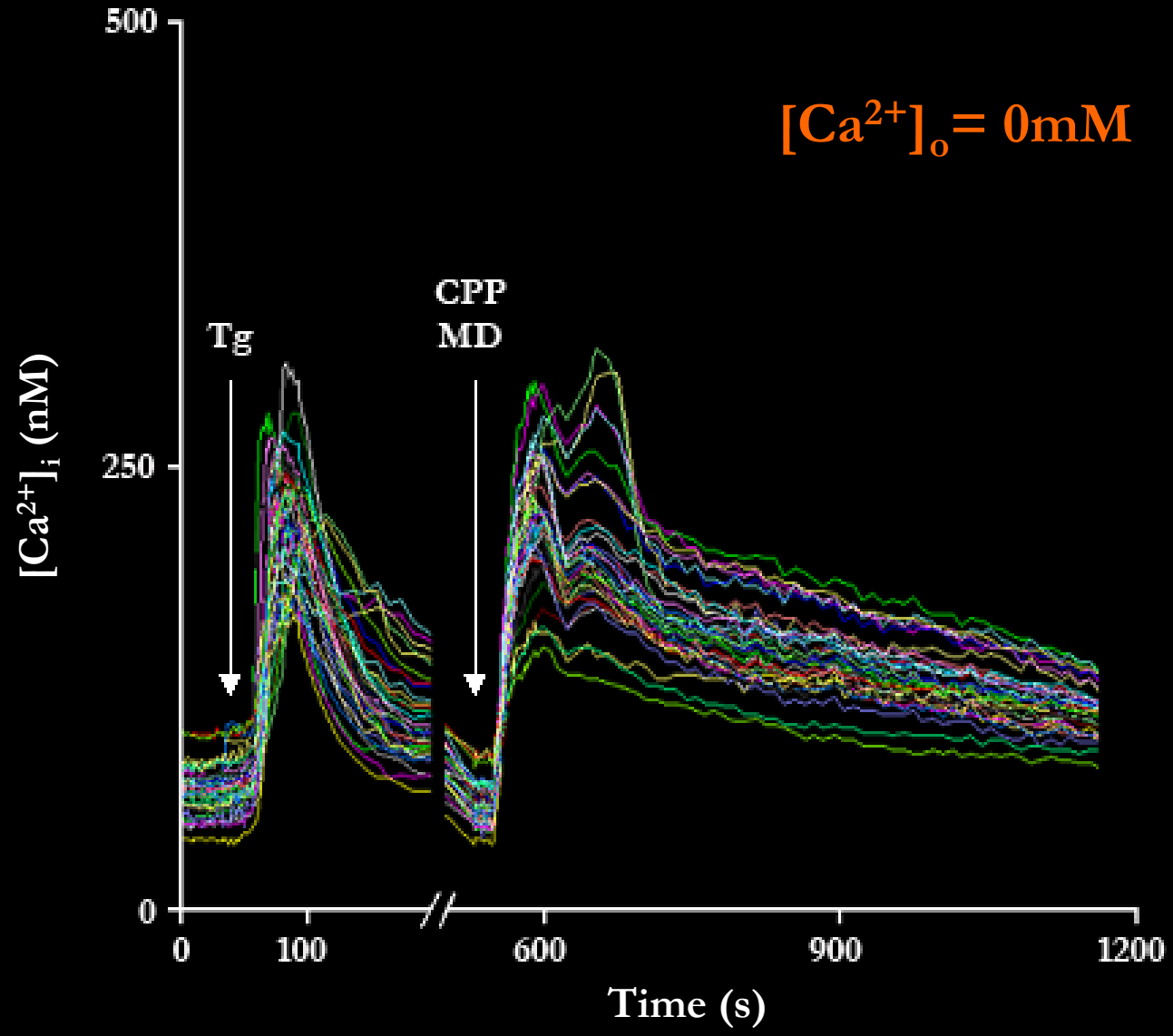
- Release of Ca^{2+} ions from CPP-calcium aggregates into the KRH buffer and consequent uptake by the cells

- Interaction of CPP with a membrane component and consequent release of bound Ca^{2+} ions within the cells

INTRACELLULAR CALCIUM HOMEOSTASIS

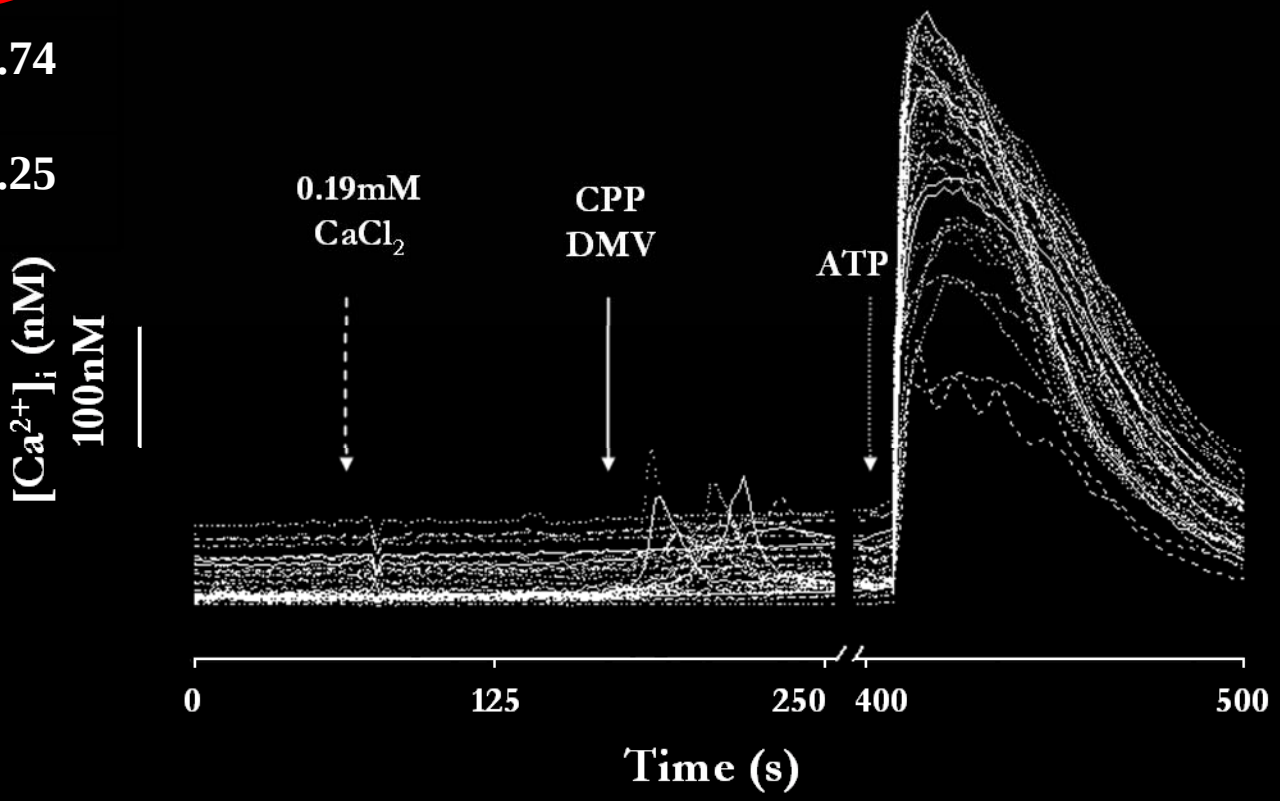


EFFECT OF CPP-MD ADMINISTRATION ON $[Ca^{2+}]_i$ AFTER Tg PRETREATMENT

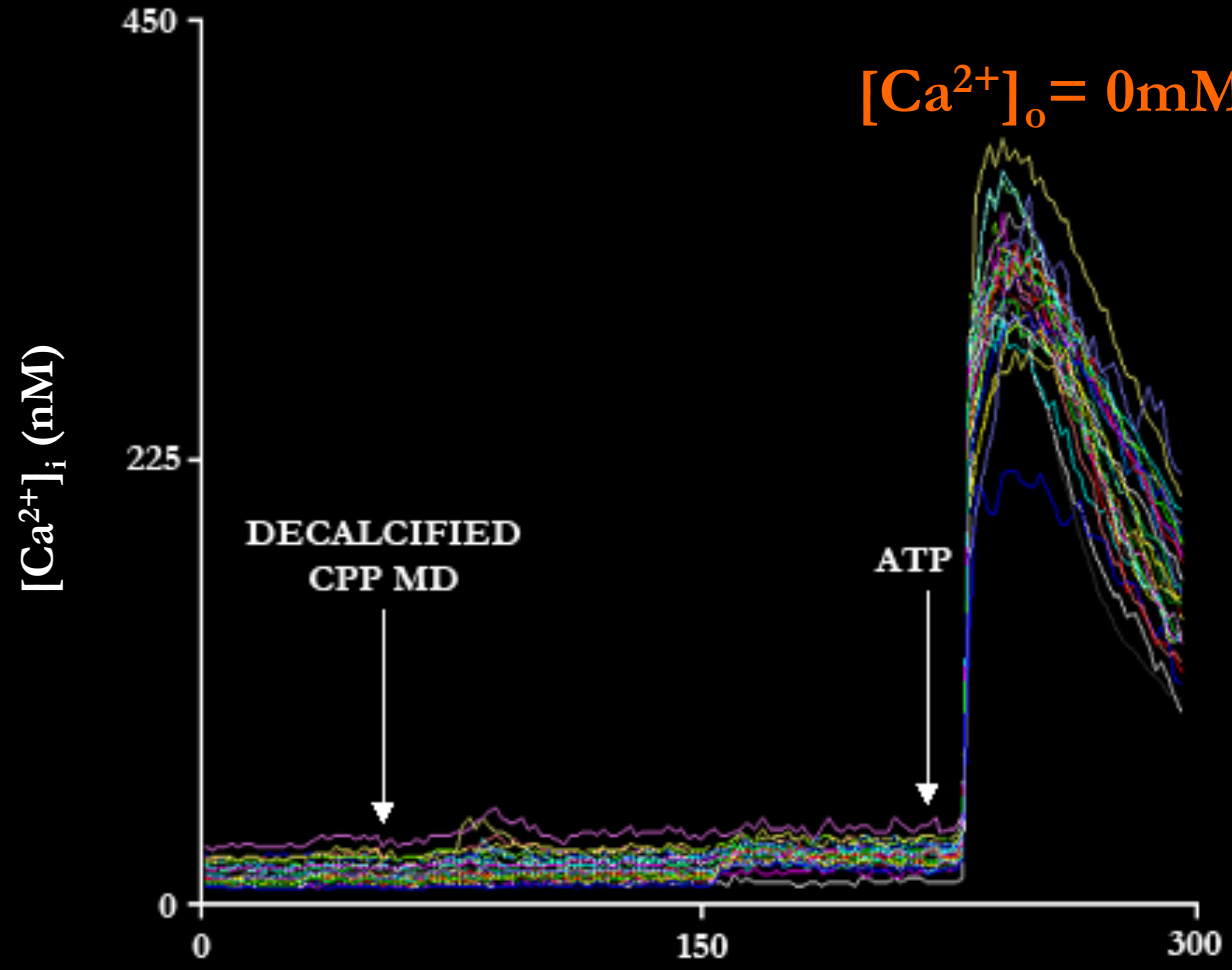


BIOLOGICAL EFFECTS OF CPP-DMV IN KRH + $CaCl_2$ 0.19mM

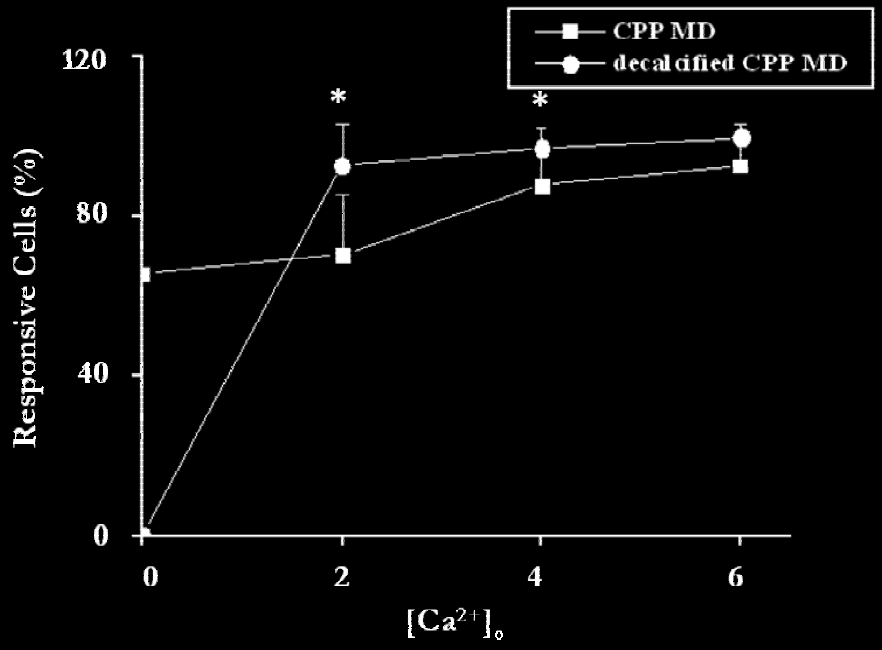
$CaCl_2$ added in KRH buffer (mM)	CPP-DMV Ca_{free}/Ca_{bound}
0	0 / 0
2	0.19 / 1.81
4	1.26 / 2.74
6	2.75 / 3.25

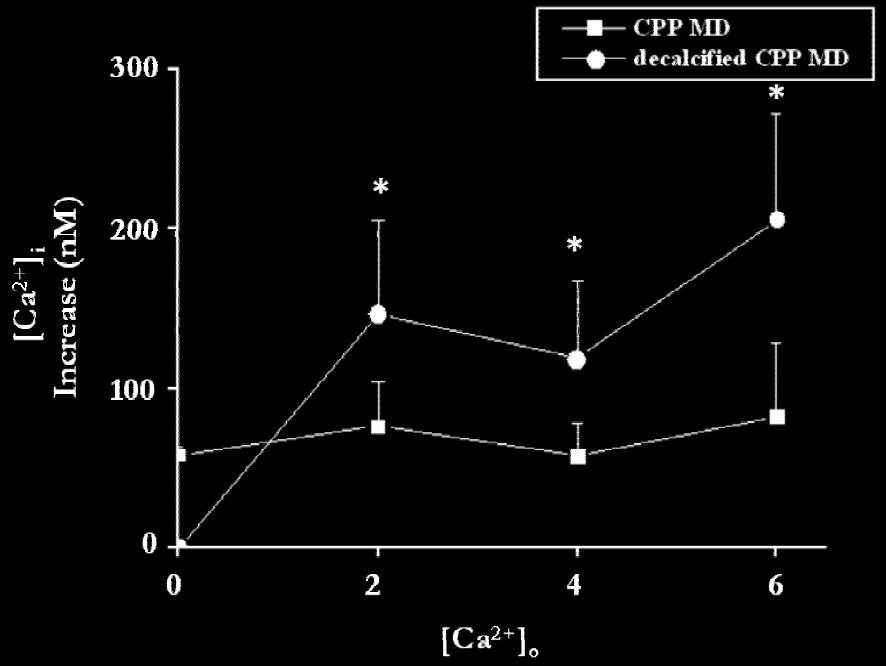


EFFECT OF DECALCIFIED CPP-MD ADMINISTRATION



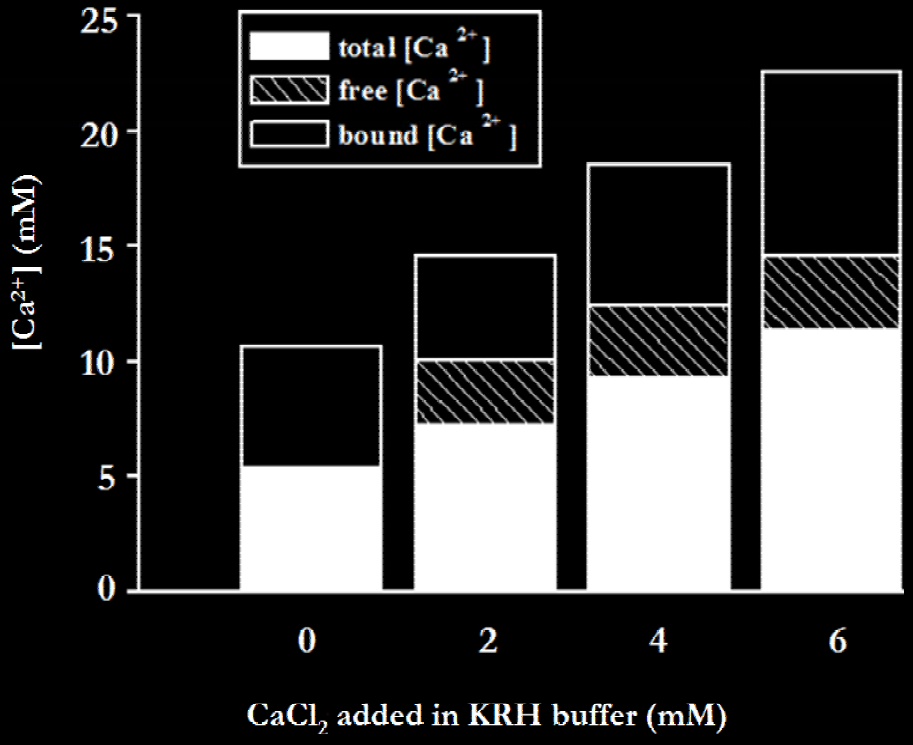
BIOLOGICAL EFFECTS OF CPP-MD AND DECALCIFIED CPP-MD



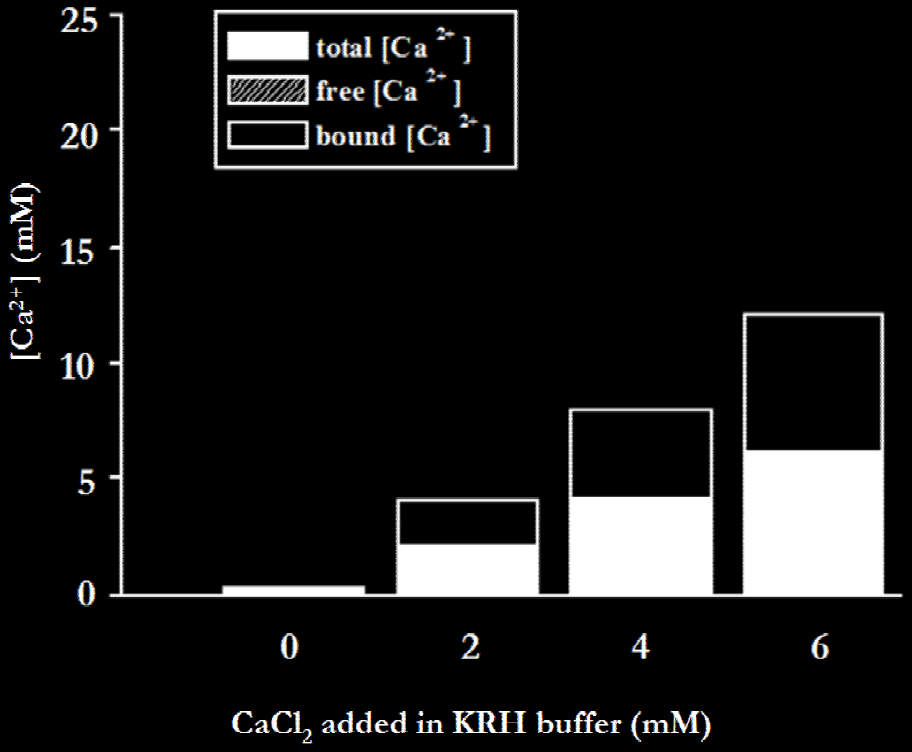


FREE Ca^{2+} TITRATION IN KRH AFTER CPP-MD AND DECALCIFIED CPP-MD SOLUBILIZATION

CPP MD



decalcified CPP MD



CONCLUSIONS

- CPP-MD induce calcium uptake in absence of extracellular calcium
- Calcium uptake induced by CPP-MD is due to entry of the ions bound to CPP itself :
  - ➔ HT-29 cells pretreated with Thapsigargin respond to CPP-MD stimulation
  - ➔ Decalcified CPP-MD does not induce calcium uptake by HT-29 cells
  - ➔ CPP-MD dissolved in calcium-free KRH buffer does not release calcium ions

CONCLUSIONS

Calcium-enriched CPP preparations (CPP-MD) display a stronger stability with respect to calcium–free CPP preparation (CPP-DMV) and a higher calcium chelating ability

Good candidate for practical applications as nutraceuticals

…IN FUTURE

Actual studies are focusing on the mechanism of action by which CPP induce calcium uptake by the cells

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