

# Le rôle du marquage au deutérium dans les applications de la diffusion neutronique à la biologie. Le laboratoire de Deutériation au sein du PSB.

Peter Timmins (ILL, Grenoble)

- Les longueurs de diffusion neutroniques - cohérente et incohérente.
- Le contraste et la variation du contraste
- Quelques exemples
- Deutériation in vivo
- Le laboratoire de deutériation et le PSB

	$b_{\text{coh}}$ $\times 10^{-12}$ cm	$f(2\theta=0)$ $\times 10^{-12}$ cm
$^1\text{H}$	-0.3742	0.28
$^2\text{H}$	0.6671	0.28
C	0.6651	1.69
N	0.940	1.97
O	0.5804	2.25
P	0.517	4.23
S	0.2847	4.50

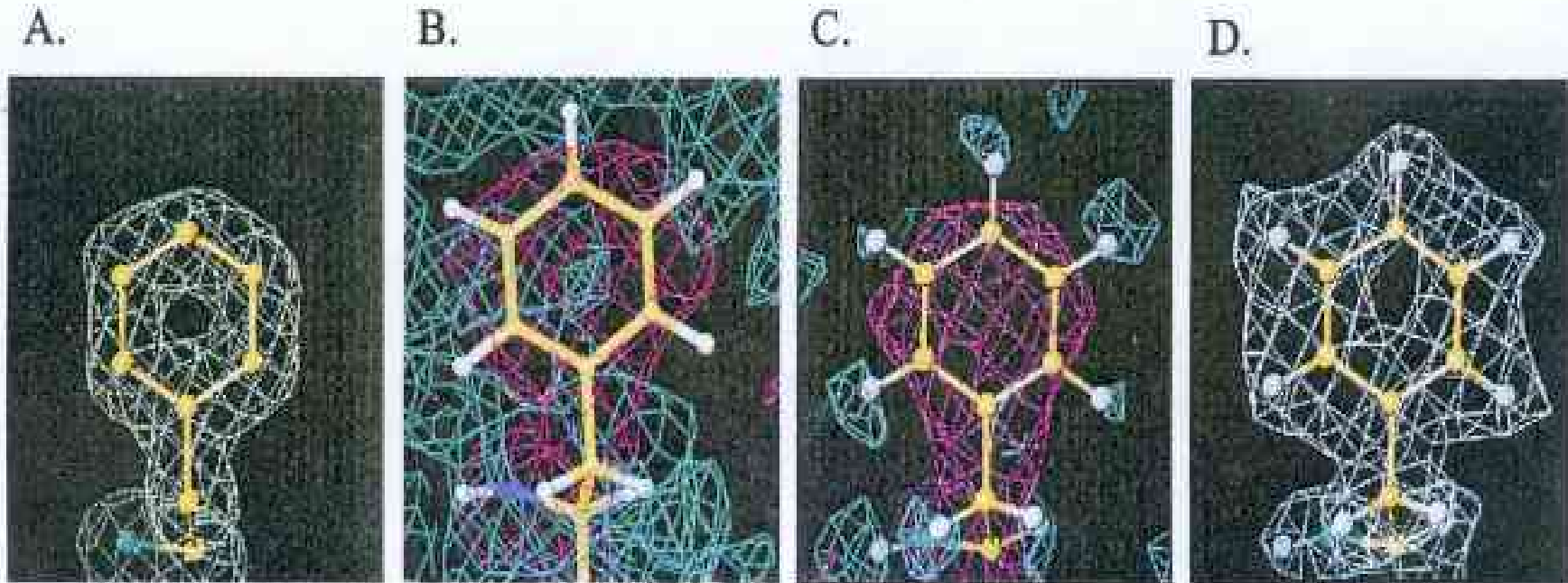
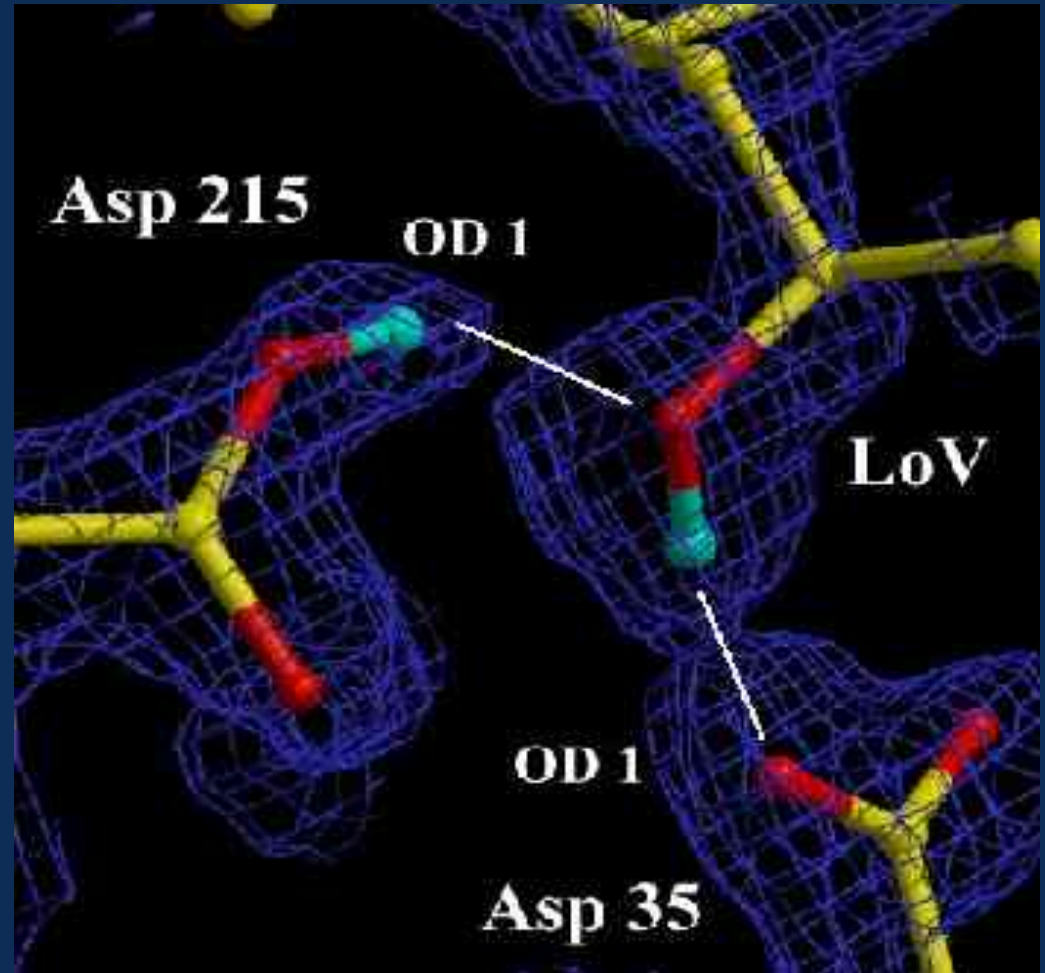
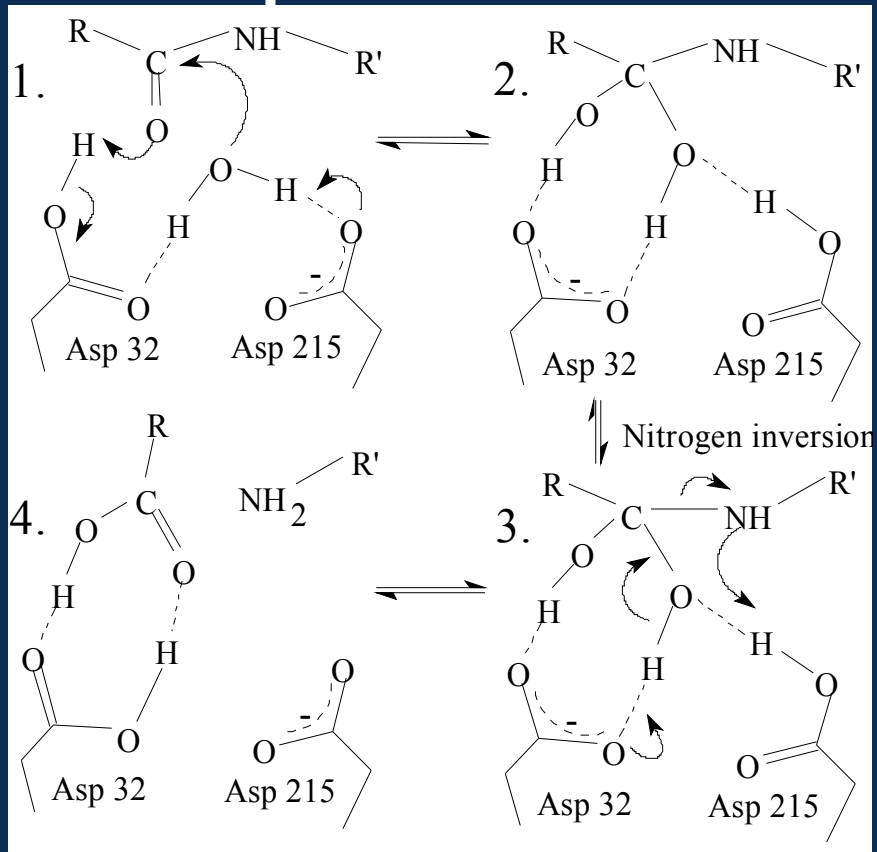


Fig. 2. Deuterium (hydrogen) atoms can be located directly as positive peaks in  $2F_o - F_c$  maps as illustrated by residue Phe-43. (A) The  $2F_o - F_c$  x-ray map of fully deuterated Mb using 6.0- to 1.5-Å data, contoured at  $+1.0 \sigma$  ( $0.84 \text{ e}/\text{Å}^3$ ). (B) A  $2F_o - F_c$  neutron map on unlabeled Mb (19) calculated to 2.0-Å resolution, with the pink map contoured at  $1.0 \sigma$  and the blue map contoured at  $-1.0 \sigma$ . (C) An  $F_2$  neutron map generated by using equivalent experimental reflections in 6.0 to 2.0 Å calculated from the current protein model except that D was replaced with H. The pink map is contoured at  $+1.0 \sigma$ ; the blue map is contoured at  $-2.0 \sigma$ . (D) The  $2F_o - F_c$  neutron map of fully deuterated Mb using 6.0- to 2.0-Å data, contoured at  $-1.0 \sigma$  ( $1.03 \text{ fermi}/\text{Å}^3$ ).

Shu et al. PNAS 1999

## Catalytic Mechanism of Aspartic Proteases

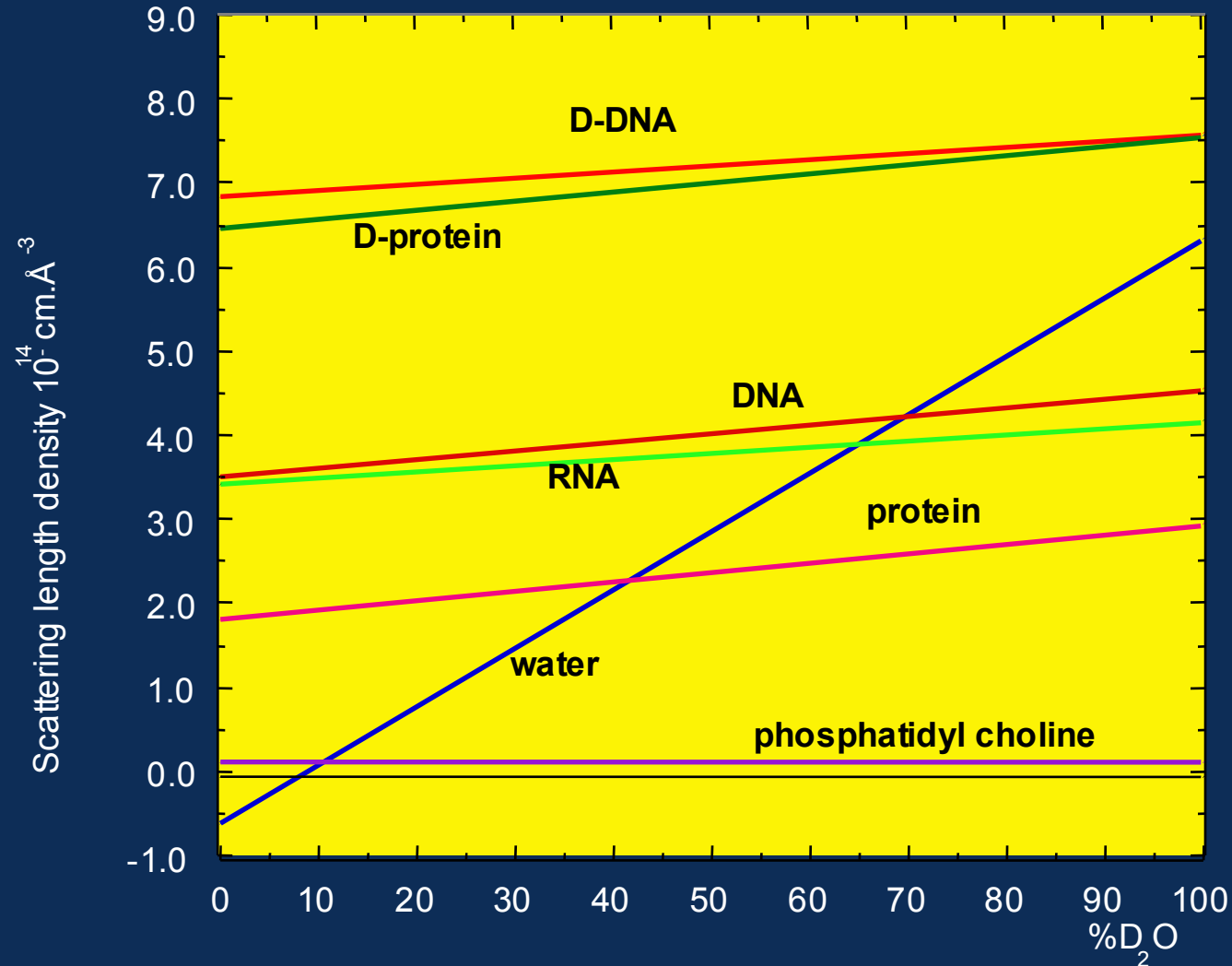


B.Veerapandian et al. ((1992) Protein Science, 1,

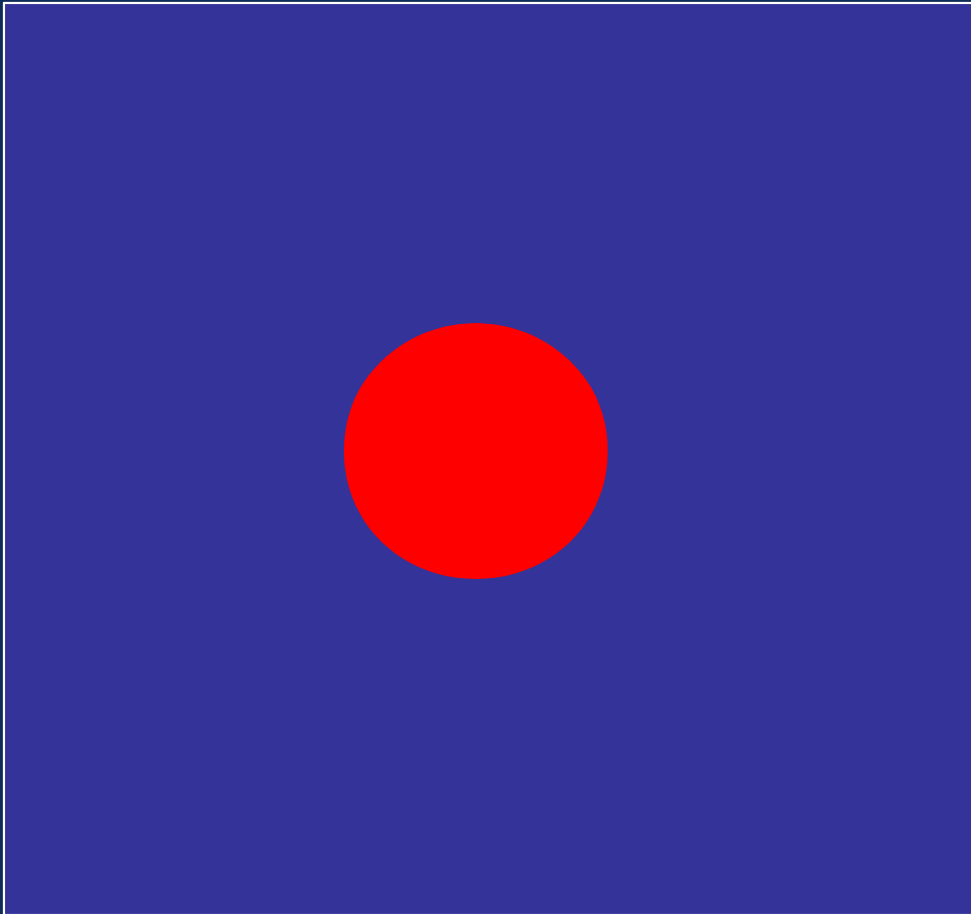
Jon Cooper, Leighton Coates(Southampton)  
Dean Myles (ILL)

# La Variation de Contraste

	N	x
$^1\text{H}$	-0.3742	0.28
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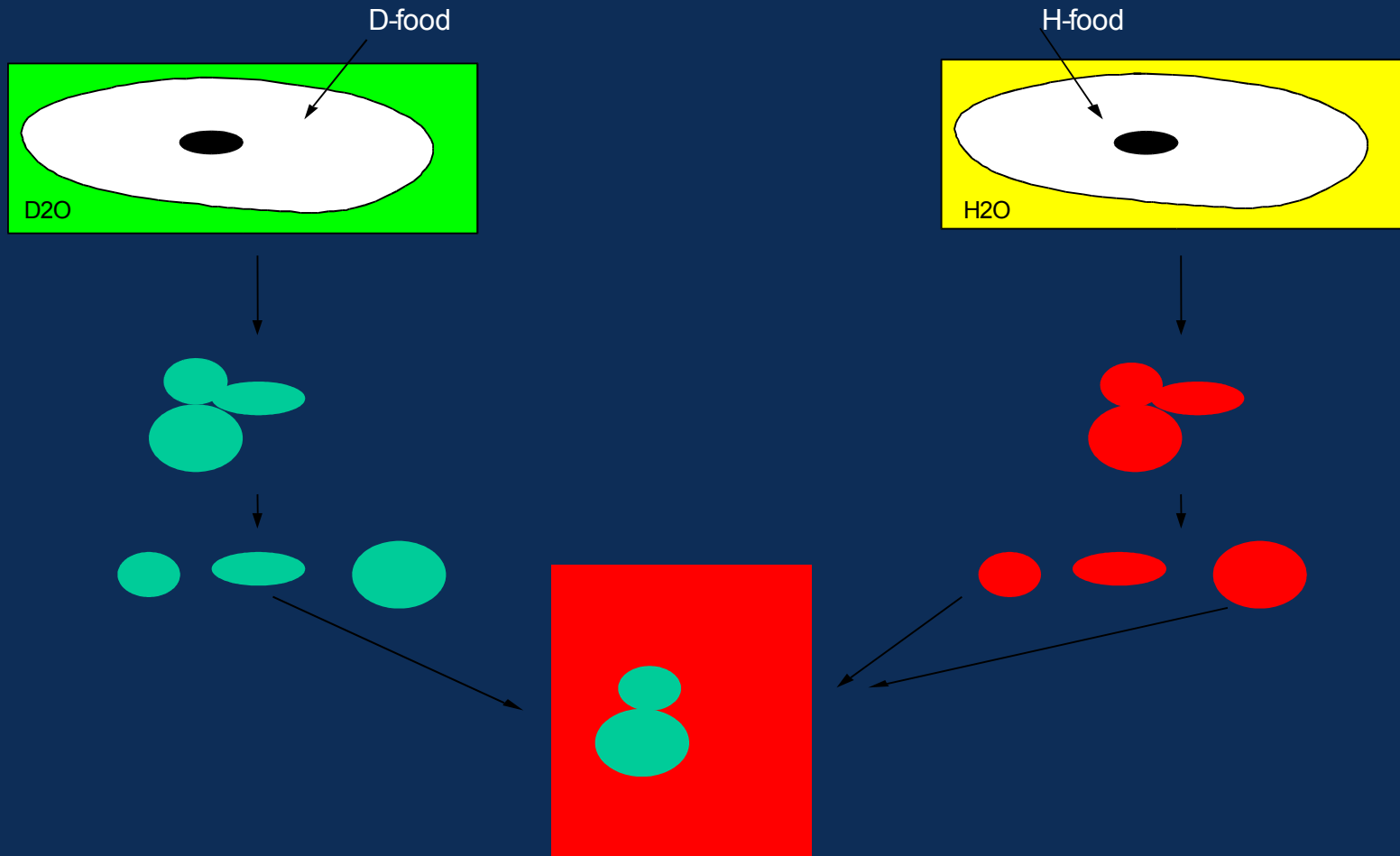


# La Variation de Contraste : le contraste naturel interne



- Les complexes acide nucléique/protéine: eg les virus
- Lipo-proteines
- Glyco-protéines

# La Variation de contraste par Deutériation in vivo

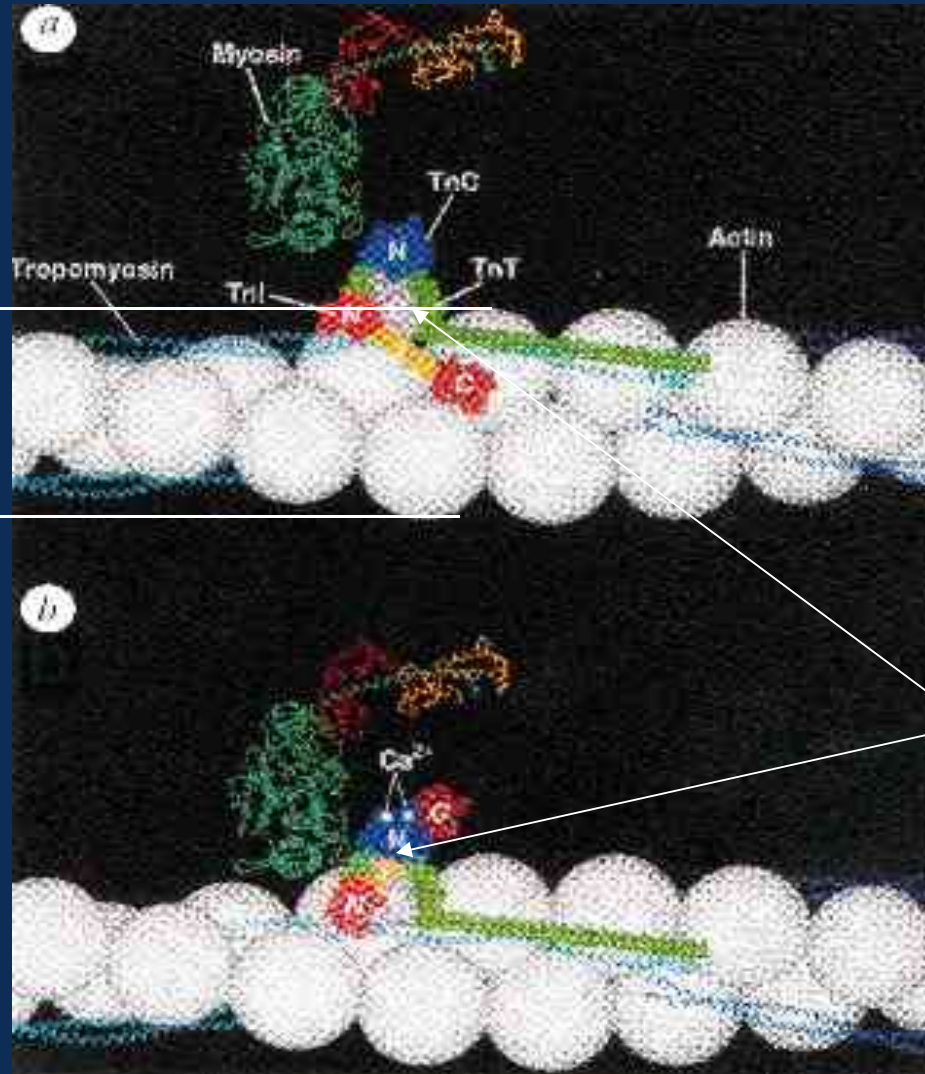


# La troponine du muscle skeletale

R. Mendelson, D. Stone (UCSF)

P. Curmi, B. King (Sydney)

P. Timmins (ILL)

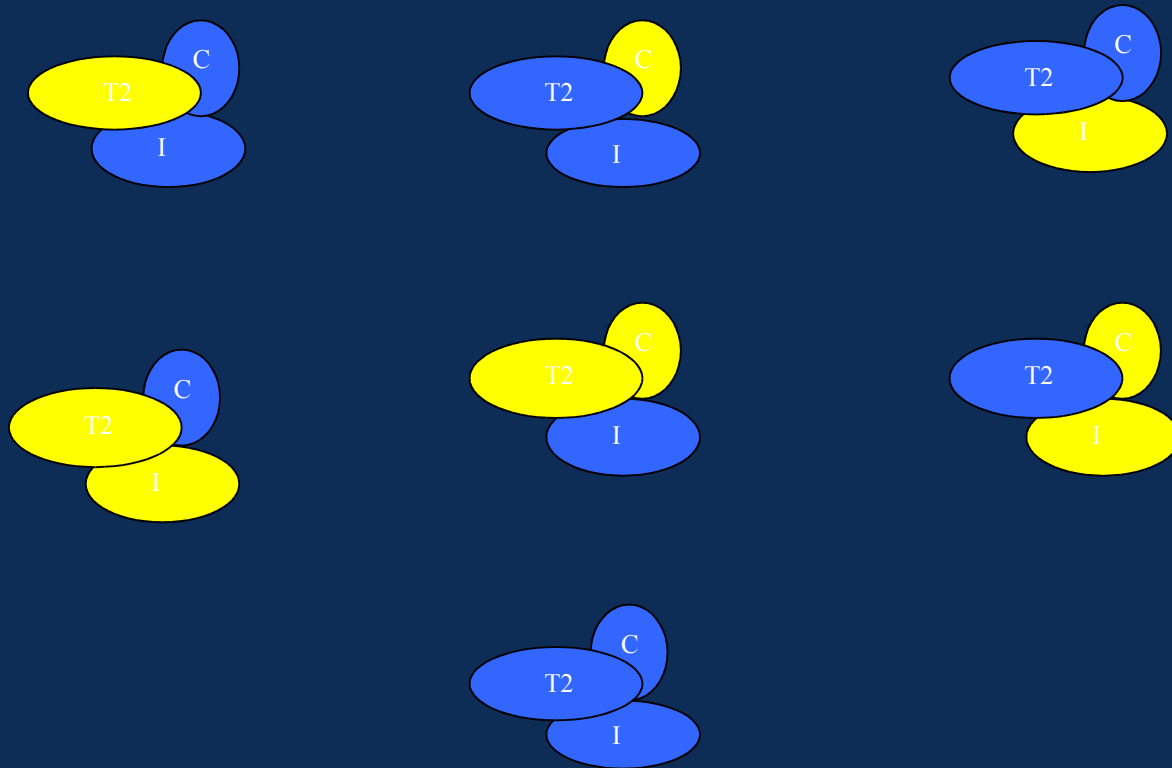


Thickness of F-actin

Separation Tm-Tm

Conformational change of Troponin

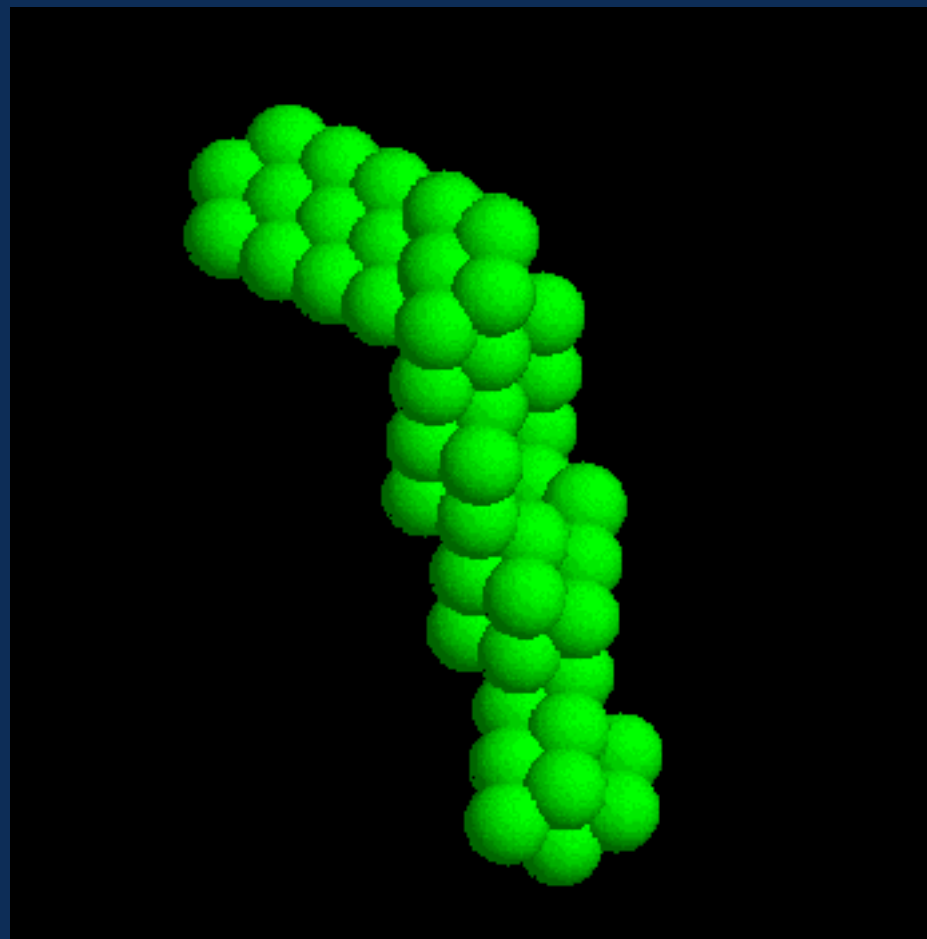
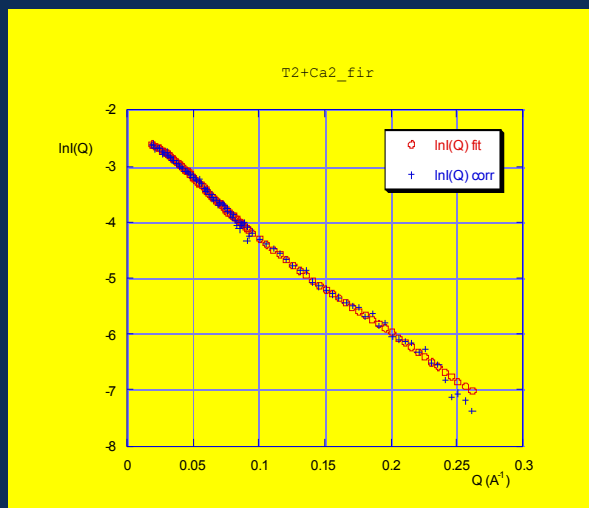
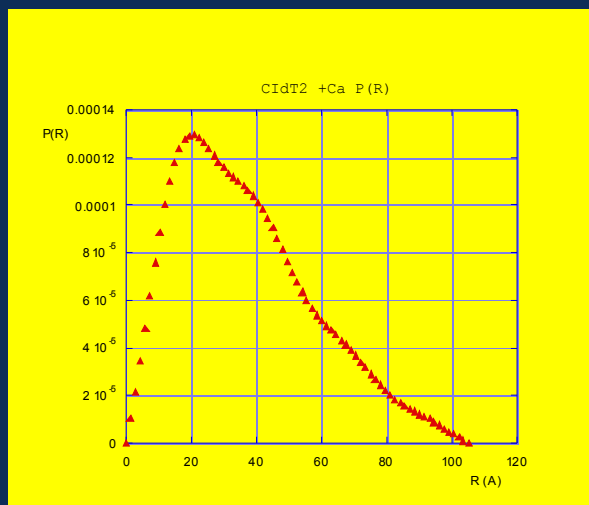
# Labelling strategy



Protonated and deuterated troponin subunits from chicken fast skeletal muscle were expressed in *Escherichia coli* as recombinant proteins. Whole troponins containing one, two or three deuterated subunits were prepared by mixing appropriately deuterated Tn subunits in the presence of urea or guanidine hydrochloride and renaturing by step-wise dialysis. **SANS measurements were carried out in a 42%  $^2\text{H}_2\text{O}$  solvent** (50 mM Hepes, 500 mM NaCl, 5 mM  $\text{MgCl}_2$ , 2 mM DTT, protease inhibitors, pH 7.6) containing either 5 mM  $\text{CaCl}_2$  (TnC regulatory sites occupied) or 10 mM EGTA (TnC regulatory sites empty).

# Dummy atom modelling CIdT2 + Ca<sup>2+</sup>

DAMMIN (Svergun et al.)



# Pourquoi le marquage au deutérium?

## Cristallographie à haute résolution:

Distinction entre H et D

Réduction de bruit du fond

## Réflexométrie

Étiquettes spécifiques

Variation de contraste

## Diffraction des fibres

Marquage spécifiques

Réduction de bruit de fond

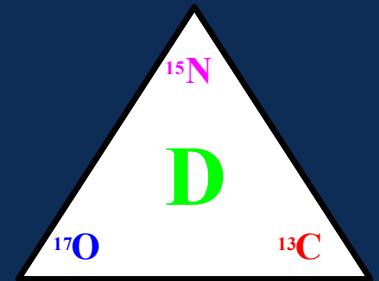
## Diffusion aux petits angles

Variation de contraste

## Diffusion inélastique

Marquage spécifique à l'hydrogène

# Le laboratoire ILL/EMBL de deutériation



- Service utilisateur
- Développement des procédures de deutériation
- Isolation des produits deutériés  
(acides aminés, glycérol...)
- la recherche interne

Par proposition avec comité de lecture internationale:

- justification scientifique
- temps nécessaire
- estimation du coût de D<sub>2</sub>O et produits deutériés

<http://www.ill.fr/deuteration/useraccessfacility.html>

# L'Equipe

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