Cécile Sicard-Roselli

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Laboratoire de Chimie Physique, Bât 350, Orsay

- **être capable de discernement lors de la lecture d'un article**
- **ne pas confondre un article/thèse séduisant(e) et une démonstration étayée, "solide"**
- **adopter une lecture active et critique**

Vendredi 2 octobre : Exposés par binôme sur les techniques « complémentaires »

Envoi du fichier de présentation le 30 septembre à : cecile.sicard@universite-paris-saclay.fr

Temps d'exposé: 15 min (10-12 diapos maximum) + 10 min de questions

Choisir un thème parmi :

1. Détection de radicaux des biomolécules par résonance paramagnétique électronique
2. Interaction protéine-ligand, protéine-ADN ou protéine-protéine par microcalorimétrie
3. Variations structurales de molécules biologiques par dichroïsme circulaire
4. Interaction protéine-ligand par spectrométrie de masse
5. Caractérisation de molécules biologiques par EXAFS ou SAXS
6. Interaction protéine-ligand ou protéine-protéine par plasmon de surface (biacore)
7. Caractérisation structurale de biomolécules par RMN

Lors de l'exposé, il faut aborder les points suivants :

1. Principe de la technique
2. Décrire l'appareillage
3. Illustrer les applications et les limites de la technique par un exemple trouvé dans un article scientifique
4. Donner une éventuelle complémentarité avec d'autres techniques d'analyse

Note qui compte pour l'examen

Pourquoi rédiger un article scientifique
Quel type de publication?

Article de recherche (étude originale)

- Diffuser les résultats d'une recherche, transmettre une information structurée
- faire avancer la connaissance au sein d'une communauté de recherche
- contribuer à l'amélioration de son dossier professionnel

Revue scientifique

- Diffuser une modélisation théorique
- Diffuser une analyse critique de l'état de la documentation scientifique dans un domaine particulier

➔ susciter le débat et la critique au sein de la communauté scientifique

Processus de publication

Ecriture (1 à plusieurs mois)

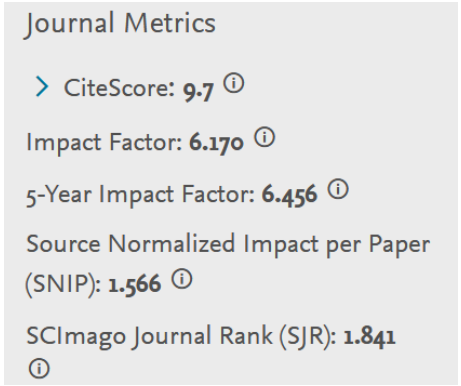
- Choix du thème
- Choix des co-auteurs
- Choix du type d'article
- Choix du journal (**support éditorial**)
- Ecriture

Processus d'acceptation (3-4 semaines minimum)

- l'article envoyé à l'éditeur
- envoyé à des relecteurs (2 à 4) désignés comme « experts » (*referees* ou *reviewers*) = anonyme
- décision: refuser l'article , exiger des révisions plus ou moins importantes (écriture, expériences, références…), accepter avec quasi aucune révision

Choix du journal (support éditorial)

- orientations théoriques du journal
- renommée (facteur d'impact ou non)
- existence ou non d'un comité de lecture
- prix de la publication
- accès (abonnement bibliothèque)



depuis quelques années: *open access*

- prix élevé
- des procédures d'expertise accélérées (parfois pas d'expertise)
- différents modèles de libre accès (certains sont légitimes, mode « archive ouverte » (ex : HAL-SHS)

Structure d'un article

Première page:

Titre, Auteurs, Abstract et Graphical abstract, Highlights

Titre
Concis et informatif
Souvent utilisé dans des systèmes de récupération d'informations
Eviter abréviations, formules

Nom d'auteurs et affiliations
Noms exacts et adresses des auteurs (mail)

- permet de contacter les auteurs
- utilisé dans des systèmes de comptages automatiques

Corresponding author

- responsable des échanges avec le journal (passés ou futurs)

Highlights
Phrases courtes représentatives de l'étude (méthode, résultats…)
Permet l'identification/référencement de l'article par des systèmes de recherche

Structure d'un article

Abstract

Pre-mRNAprocessing protein 40 (Prp40) is a nuclear protein that has a role in pre-mRNA splicing. Prp40 possesses two leucine-rich nuclear export signals, but little is known about the function of Prp40 in the export process. Another protein that has a role in protein export is centrin, a member of the EF-hand superfamily of Ca^{2+}-binding proteins. Prp40 was found to be a centrin target by yeast-two-hybrid screening using both Homo sapiens centrin 2 (Hscen2) and Chlamydomonas reinhardtii centrin (Crcen). We identified a centrin-binding site within H. sapiens Prp40 homolog A (HsPrp40A), which contains a hydrophobic triad W1L4L8 that is known to be important in the interaction with centrin. This centrin-binding site is highly conserved within the first nuclear export signal consensus sequence identified in Saccharomyces cerevisiae Prp40. Here, we examine the interaction of HsPrp40A peptide (HsPrp40Ap) with both Hscen2 and Crcen by isothermal titration calorimetry. We employed the thermodynamic parameterization to estimate the polar and apolar surface area of the interface. In addition, we have defined the molecular mechanism of thermally induced unfolding and dissociation of the Crcen-HsPrp40Ap complex using two-dimensional infrared correlation spectroscopy. These complementary techniques showed for the first time, to our knowledge, that HsPrp40Ap interacts with centrin in vitro, supporting a coupled functional role for these proteins in pre-mRNA splicing.

Primary liver cancer or hepatocellular carcinoma (HCC) is an aggressive tumor with poor prognosis. Olea europaea L. commonly called olive contains diverse phytochemical constituents that have preventive effects against different diseases. The purpose of this investigation was to study the chemopreventive effect of aqueous extract of Olea europaea L. (AOE) against diethylnitrosamine (DEN)-induced HCC in rats. Olive-treated rats were pretreated with AOE intragastrically at three different doses two weeks prior to DEN injection that continued until 8 weeks. At three different time points, blood samples were collected for biochemical analysis. In the
present investigation, a significant decrease in serum biomarkers of liver damage and cancer, including alfafetoprotein (AFP), gamma glutamyl transpeptidase (GGT), alanine transaminase (ALT), and aspartate transaminase (AST) were observed in AOE supplemented animals when compared to DEN-treated rats. In addition, AOE counteracted DEN-induced hepatic oxidative stress in rats illustrated by the restoration of reduced glutathione (GSH) and the reduction of lipid peroxidation in HCC rats. Furthermore, AOE prevented increase of relative liver weight as a prognostic marker in HCC. Finally, histopathological investigation in the liver sections confirmed the preventive effect of AOE against DEN-induced HCC. To conclude, olive has a preventive effect against DEN-induced hepatocarcinogenesis in rats.

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To conclude, olive has a preventive effect against DEN-induced hepatocarcinogenesis in rats.

- contexte
- objectif/but/motivation de l'étude
- méthodologie (démarche adoptée et ressources mobilisées)
- résultats
- conclusion

Structure d'un article

Graphical abstract

- "résumer" l'article
- permettre d'identifier le message important
- donner envie de lire l'article

Highlights

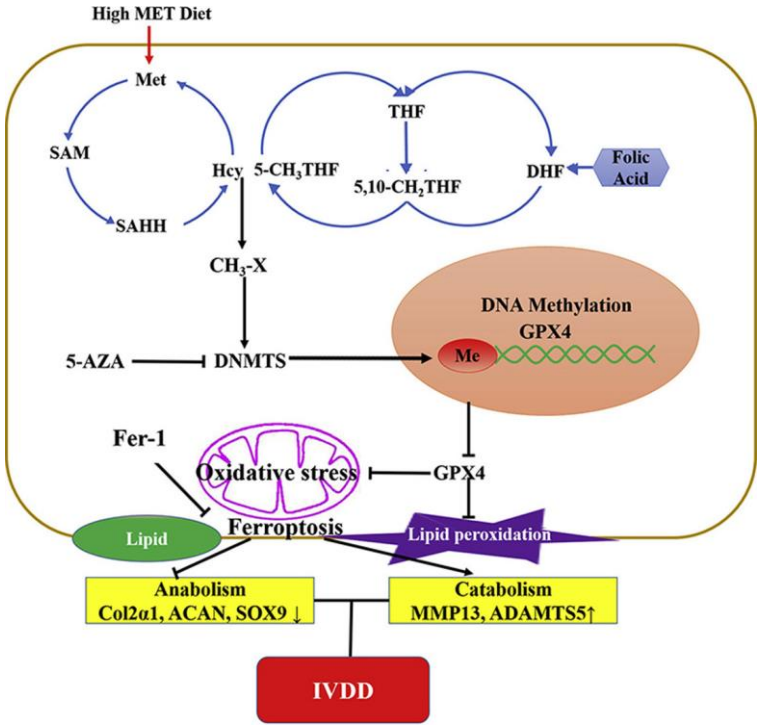
- 3 à 5 courtes phrases ou "idées clés": nouveautés des résultats, les méthodes utilisées
- Aide à l'identification de l'article par des moteurs de recherche (Nowadays, machines read your work just as often as humans do!)
- rendre l'article + visible dans et hors la communauté habituelle ➔ nouvelles collaborations?

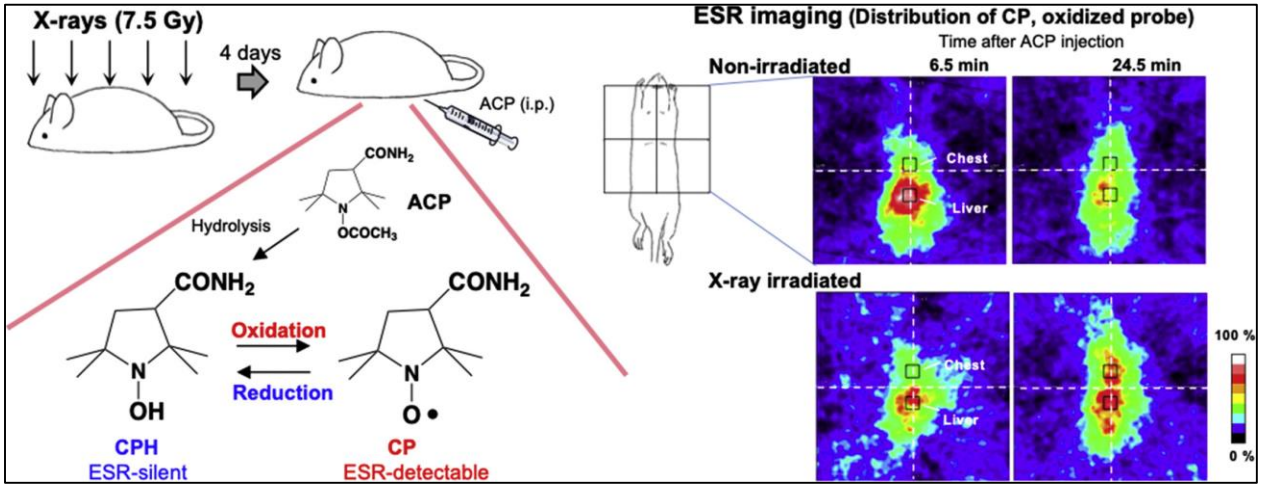
Abstract/highlights

Homocysteine induces oxidative stress and ferroptosis of nucleus pulposus via enhancing methylation of GPX4

Highlights

- Homocysteine aggravates oxidative stress and increases ferroptosis of nucleus pulposus cells, which lead to intervertebral disc degeneration.
- Homocysteine can promote the expression of methylase and upregulate the methylation of GPX4.
- Inhibiting methylation caused by homocysteine can alleviate oxidative stress and ferroptosis, and rescue nucleus pulposus degeneration





***In vivo* ESR imaging of redox status in mice after X-ray irradiation, measured by acyl-protected hydroxylamine probe, ACP**

Highlights

• X-ray irradiation induces oxidative stress monitored non-invasively in mice.

• In vivo ESR imaging with probe ACP demonstrates redox alterations in liver and chest.

• X-ray-induced oxidative stress contributes to microsome deactivation.

Abstract/graphial abstract

Primary liver cancer or hepatocellular carcinoma (HCC) is an aggressive tumor with poor prognosis. Olea europaea L. commonly called olive contains diverse phytochemical constituents that have preventive effects against different diseases. The purpose of this investigation was to study the chemopreventive effect of aqueous extract of Olea europaea L. (AOE) against diethylnitrosamine (DEN)-induced HCC in rats. Olive-treated rats were pretreated with AOE intragastrically at three different doses two weeks prior to DEN injection that continued until 8 weeks. At three different time points, blood samples were collected for biochemical analysis. In the
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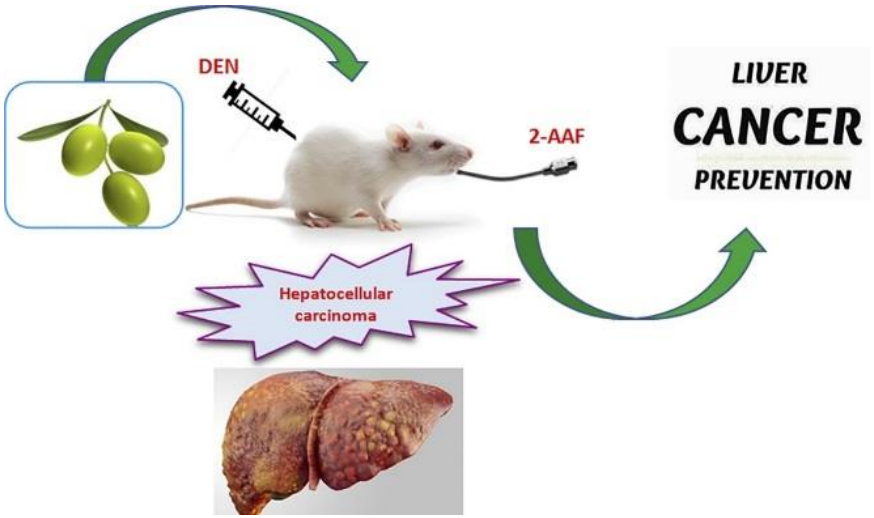

Shirazi et al 2018

Structure d'un article

IMRAD:
Introduction, Method, Results and Discussion (supplementary)

INTRODUCTION: contexte et la problématique

Contexte

- élément essentiel (surtout si lectorat hors du domaine)
- brève description (deux ou trois paragraphes) de l'univers de la recherche décrite, des enjeux

Problématique:

- synthèse de la bibliographie actuelle du sujet ou nécessaire à l'étude
- justifie l'objectif de l'article et introduit son cadre théorique

- méthodologie et résultats

INTRODUCTION

In the nuclei of eukaryotic cells, precursor mRNA (pre-mRNA) undergoes splicing, a process in which the introns are removed from the primary transcripts and the exons are combined to generate mRNA. This process is carried out by the spliceosome, a multi-protein complex composed of U1, U2, U4, U5, and U6 small nuclear ribonucleoproteins and numerous other proteins (1). Recently, the structure of the spliceosome in *Schizosaccharomyces pombe* was obtained using cryogenic electron microscopy (2). Pre-mRNA processing protein 40 (Prp40), first identified in *Saccharomyces cerevisiae*, has an essential role in the initiation step of pre-mRNA splicing (3–5). In higher eukaryotes, such as *Homo sapiens* and *M. musculus*, there are two putative homologs of Prp40: homolog A (Prp40A) and homolog B (Prp40B), also known as HYPA/FBP11 and HYPC, respectively (6–13). Based on phylogenetic analysis, Prp40A is more closely related to *S. cerevisiae* Prp40 (*Sc*Prp40) than to Prp40B (9). Both homologs contain two WW domains followed by six tandem FF domains (FF_1–FF_6), which mediate Prp40-target interactions. The WW-domain-mediated interactions of *H. sapiens* Prp40A (*Hs*Prp40A) and Prp40B (*Hs*Prp40B) are also implicated in genetic disorders such as Huntington's disease and Rett syndrome (7,8,14,15). In addition to the WW and FF domains, Murphy et al. (16) found two nuclear export signals (NESs) within *Sc*Prp40 whose sequences are $_{274}$LKELREYLNGI$_{284}$ (NES_1) and $_{340}$LQNKLNELRL$_{349}$ (NES_2). Mutations in NES_1 or both NES_1 and NES_2 result in deficient in vivo splicing, suggesting that these NES sequences are required for efficient pre-mRNA splicing. Moreover, little is known about the function of Prp40 in the export process.

Centrin has been found to play a role in protein and mRNA export. Centrin is a member of the EF-hand superfamily of Ca^{2+}-binding proteins (17–21), and in *H. sapiens*, it has four isoforms: centrin 1 (*Hs*cen1), centrin 2 (*Hs*cen2), centrin 3 (*Hs*cen3), and the pseudogene centrin 4 (*Hs*cen4) (22–26). *Hs*cen1 is localized in male germ cells, neurons, and ciliary cells (23,27). In contrast, *Hs*cen2 and *Hs*cen3 are ubiquitously expressed in all somatic cells. *Hs*cen1 and *Hs*cen2 have 80% sequence identity, yet exhibit different affinities for Ca^{2+}, whereas *Chlamydomonas reinhardtii* centrin (*Cr*cen) shares ~70% sequence identity with *Hs*cen1 and *Hs*cen2 (26,28).

Centrin has two independent domains, each containing two EF-hands that are composed of helix-loop-helix motifs (26,28–32). Ca^{2+} binding by centrin induces an open conformation, allowing buried hydrophobic residues to be exposed to the solvent. In general, centrins have different relative affinities for Ca^{2+} (29,33–35).

In *H. sapiens*, *Hs*cen2 has been localized to the centriole and the nucleus (19,36,37). In the centrosome, *Hs*cen2 has many targets, such as Sfi1, a 1242-aa protein with 23 tandem centrin-binding sites (CBSs). The centrin-Sfi1 complex was found to be essential for centriole duplication (34,38). *H. sapiens* POC5 (*Hs*POC5) is localized to the distal end of the centriole and contains only three CBSs. *Hs*POC5 has been associated with the assembly of the distal half of centrioles and is required for centriole elongation (39).

Within the nucleus, *Hs*cen2 participates in DNA nucleotide excision repair via its interaction with Xeroderma pigmentosum group C (XPC) and RAD23 homolog B. This complex functions as a main damage sensor in global genome nucleotide excision repair (40). *Hs*cen2 enhances the affinity of XPC for the damaged DNA segment (40–42). In addition, *Hs*cen2 is a component of the TREX-2 complex, which is vital for mRNA export (19–21,43–45).

The basis for centrin's target selectivity is the consensus sequence $W_1xxL_4xxxL_8$ (where x represents any residue), also known as the hydrophobic triad $W_1L_4L_8$. This sequence has been found in all available centrin-target complex structures (Fig. 1 *A*) (29,33–35,43,46). In this hydrophobic triad, W_1 is a well conserved residue among centrin targets, L_4 can usually be substituted with another hydrophobic residue such as phenylalanine or tryptophan, and L_8 is the least conserved residue. High-resolution structural studies of *Hs*cen2 in complex with XPC or Sfi1, as well as the ternary Sac3-Cdc31-Sus1 complex, have W_1 interacting within a hydrophobic pocket within the *Hs*cen2 (or Cdc31, a yeast homolog) C-terminal domain (33,34,43,47).

Prp40 was first found to be a potential centrin target in yeast-two-hybrid (Y2H) screening assays using both *Hs*cen2 and *Cr*cen. Here, we examine, to our knowledge, a novel centrin target, *Hs*Prp40A peptide (*Hs*Prp40Ap) interaction. In this work, the location of the CBS in *Hs*Prp40A is validated experimentally. We describe fragment-based peptide design to determine the interaction of *Hs*Prp40Ap with different centrins using a combination of isothermal titration calorimetry (ITC) and two-dimensional infrared (2D IR) correlation spectroscopy (35). The first allows the determination of the thermodynamics governing binding, whereas the second determines the molecular requirements of binding and stability.

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La méthodologie et les résultats

- Présentation de la méthode de recueil de données
- Description détaillée des instruments
- Présentation de la préparation des échantillons (nombre de sujets; principales caractéristiques de distribution)

➔ Aide à comprendre l'expérience réalisée, son interpretation

➔ Aide à évaluer la validité du résultats

Recombinant protein expression, isolation, and purification

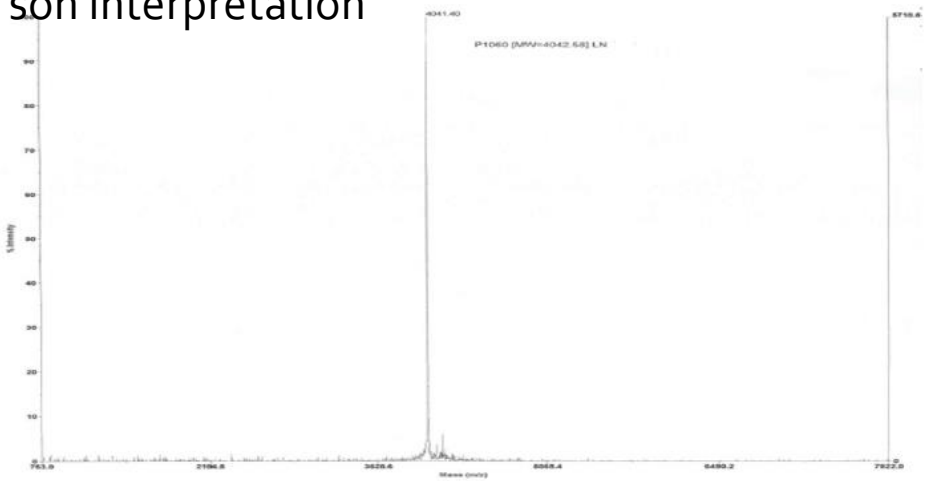
Expression and purification of recombinant centrin were performed as described in Sosa et al. (35) and Pastrana-Ríos et al. (26,35).

Y2H screen

A Y2H screen for full-length and single-domain constructs of *H. sapiens* and *C. reinhardtii* centrin was performed at the Yeast Resource Center (48). One of the major validated hits in the screen was Prp40.

*Hs*Prp40A peptide

*Hs*Prp40Ap consisting of the amino acid sequence Ac-$_{524}$KQLRKRNWE ALKNILDNMANVTYSTTWSEAQQY$_{556}$-$CONH_2$ was purchased from Bio-Synthesis. (Lewisville, TX) as a custom synthetic product. MS and HPLC results are shown in Fig. S1 and were performed by Bio-Synthesis to validate the molecular mass (4041.40 *m/z*) and the purity (>94%) of the desired peptide. To remove the trifluoroacetic acid from the peptide, the sample was subjected to repeated lyophilizations in the presence of 0.1 N HCl followed by an exhaustive dialysis against 50 mM HEPES, 150 mM NaCl, 4 mM $CaCl_2$, and 4 mM $MgCl_2$ at pH 7.4. The *Hs*Prp40Ap molar extinction coefficient is $\varepsilon = 13{,}980\ M^{-1}\ cm^{-1}$ at 280 nm. The calibration curve of *Hs*Prp40Ap for different concentration ranges is shown in Fig. S2.



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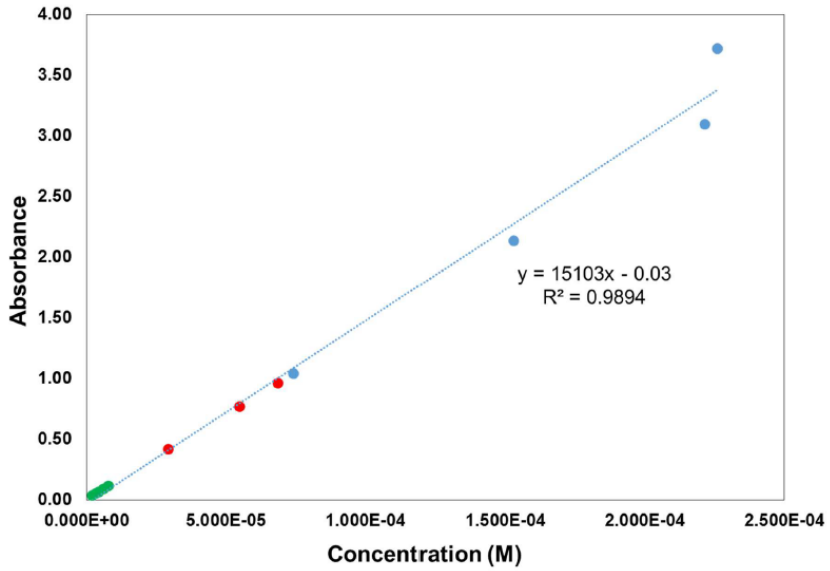

Fig. S2. UV Calibration curve for *Hs*Prp40Ap at 280 nm. Three different data sets were used to generate the calibration curve at different concentration ranges. Each data set is shown with respective closed circle color (green, red, and blue) for *Hs*Prp40Ap. For initial evaluations of accuracy in concentration determination (green closed circles) highly diluted samples were prepared. The working stocks (red closed circles) from which diluted samples were prepared for the ITC experiments. The absorbance data (blue closed circles) were normalized due to the smaller path-length of 0.2 cm used for high peptide concentrations.

La méthodologie et les résultats

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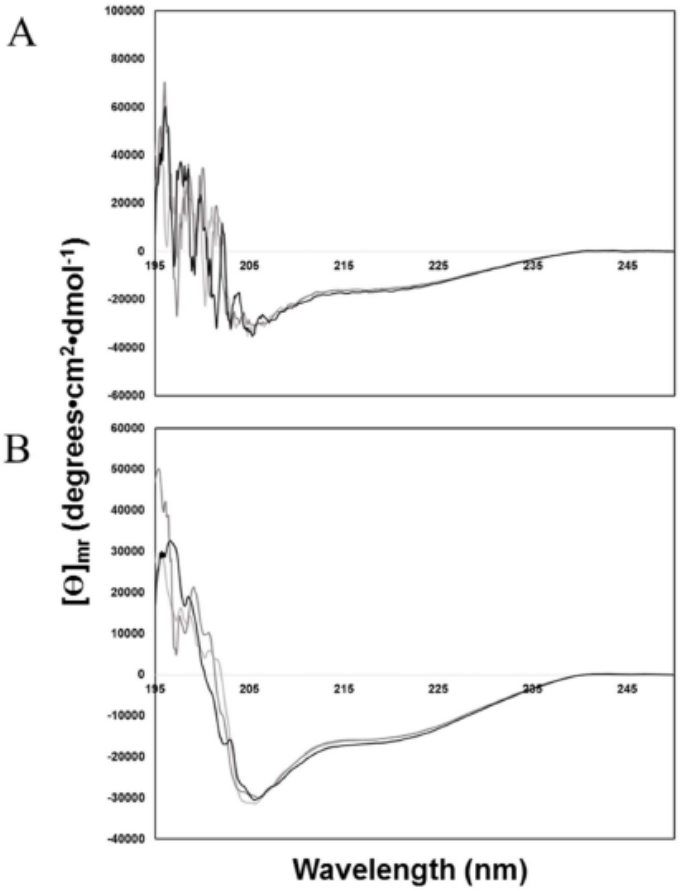
CD spectroscopy

*Hs*Prp40Ap (12 μM) in 8 mM HEPES, 50 mM NaCl, 2 mM $CaCl_2$, and 2 mM $MgCl_2$ at pH 7.4 was used to acquire far-UV CD spectra on a Jasco J-810 spectropolarimeter (Jasco, Tokyo, Japan). Five scans within the spectral range of 250–195 nm were collected at a scan rate of 200 nm/min, a response time of 2 s, a bandwidth of 2 nm, and a temperature of 25°C. Baseline correction was performed over the spectral range of 250–240 nm. The CD absorbance was converted to mean residue molar ellipticity to analyze the secondary structure contribution of the peptide. The experiment was carried out in triplicate ($n = 3$), and the results are shown in Fig. S3.



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ITC

Titrations were carried out using a VP-ITC microcalorimeter from MicroCal (Northampton, MA). Protein samples were exhaustively dialyzed separately against the desired buffer: 50 mM HEPES, 150 mM NaCl, 4 mM $CaCl_2$, and 4 mM $MgCl_2$ at pH 7.4. The protein and peptide concentrations were determined using a Jasco model V-560 UV/Vis spectrophotometer. The calculated molar extinction coefficient (ε) was the same for *Hs*cen1, *Hs*cen2, and *Cr*cen: $\varepsilon = 1490\ M^{-1}\ cm^{-1}$ at 280 nm. In a typical experiment, 6–10 μM *Hs*Prp40Ap within the sample cell was titrated with 36–144 μM centrin by automatic injection with volumes ranging from 5 to 10 μL. The first injection was set at 2 μL and was ignored in the final data analysis. All of the solutions were degassed for at least 10 min before use.

The ITC data were fitted with a one-binding-site model, using MicroCal Origin software (Northampton, MA). The values for the change in enthalpy of binding (ΔH_B), the change in entropy of binding (ΔS_B), the association constant (K_a), and the stoichiometry of binding (n) were determined. The change in Gibbs free energy (ΔG_B) was calculated using Eq. 1:

$$\Delta G_B = -RT \quad \ln K_a. \qquad (1)$$

For a one-binding-site model, the C value was calculated using Eq. 2:

$$C = \frac{[P]_t}{K_d}, \qquad (2)$$

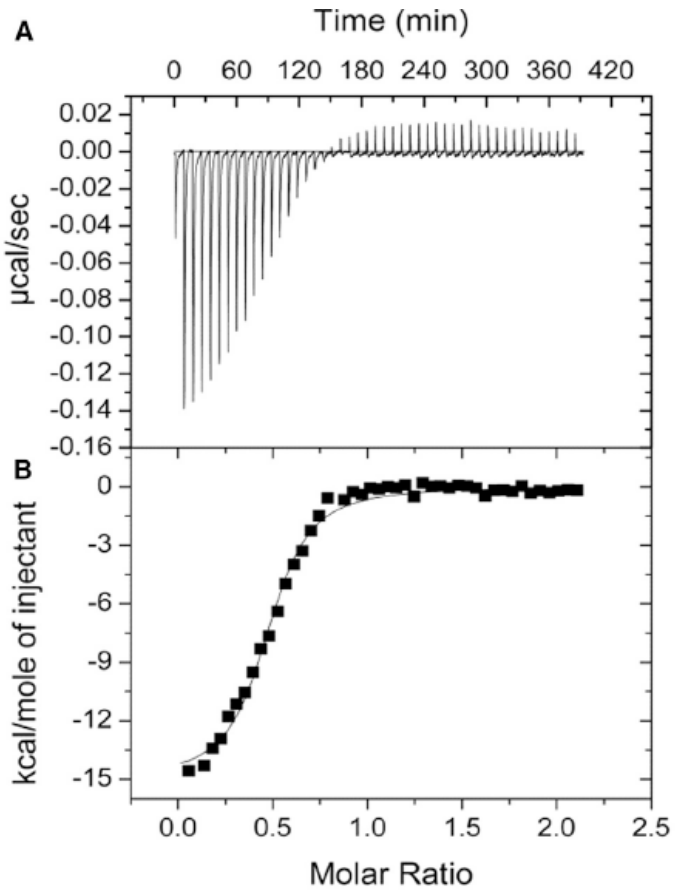

FIGURE 2 Binding isotherm of the titration of *Hs*Prp40Ap by *Hs*cen2 at 25°C. (*A*) Power data and (*B*) integrated enthalpy are fitted to a one-binding-site model.

Fourier transform IR spectroscopy

^{13}C-*Cr*cen-*Hs*Prp40Ap complex (1:1 molar ratio) was prepared under the desired buffer conditions (25 mM HEPES, 150 mM NaCl, 2 mM $CaCl_2$, and 2 mM $MgCl_2$ at pD 6.6) using D_2O after complete hydrogen-to-deuterium (H→D) exchange via freeze drying as per Sosa et al. (35). ^{13}C-*Cr*cen-*Hs*Prp40Ap complex (8.25 mg/mL) was deposited onto a round 49 × 4 mm custom-milled CaF_2 window with a fixed pathlength of 40 μm (Spectral Systems, Hopewell Junction, NY); the reference cell contained buffer only. Both cells were set in a custom dual-chamber cell holder. The temperature within the cell holder was controlled with a Neslab RTE-740 refrigerated circulating bath (Thermo Electron, Newington, NH). The temperature was monitored with a thermocouple positioned in close contact with the cell. Spectral data acquisition was performed at the desired preset temperature. Once the temperature of the cell was reached, 10 min was allowed for thermal equilibrium. The temperature range studied was 5–90°C with 5°C intervals. The instrument used was a Jasco Fourier transform (FT-IR) spectrophotometer model 6200 equipped with an MCT detector, a sample shuttle, and an interface. In this experiment, 640 scans were co-added, apodized with a triangular function, and Fourier transformed to provide a resolution of 4 cm^{-1} with the data encoded every 2 cm^{-1}.

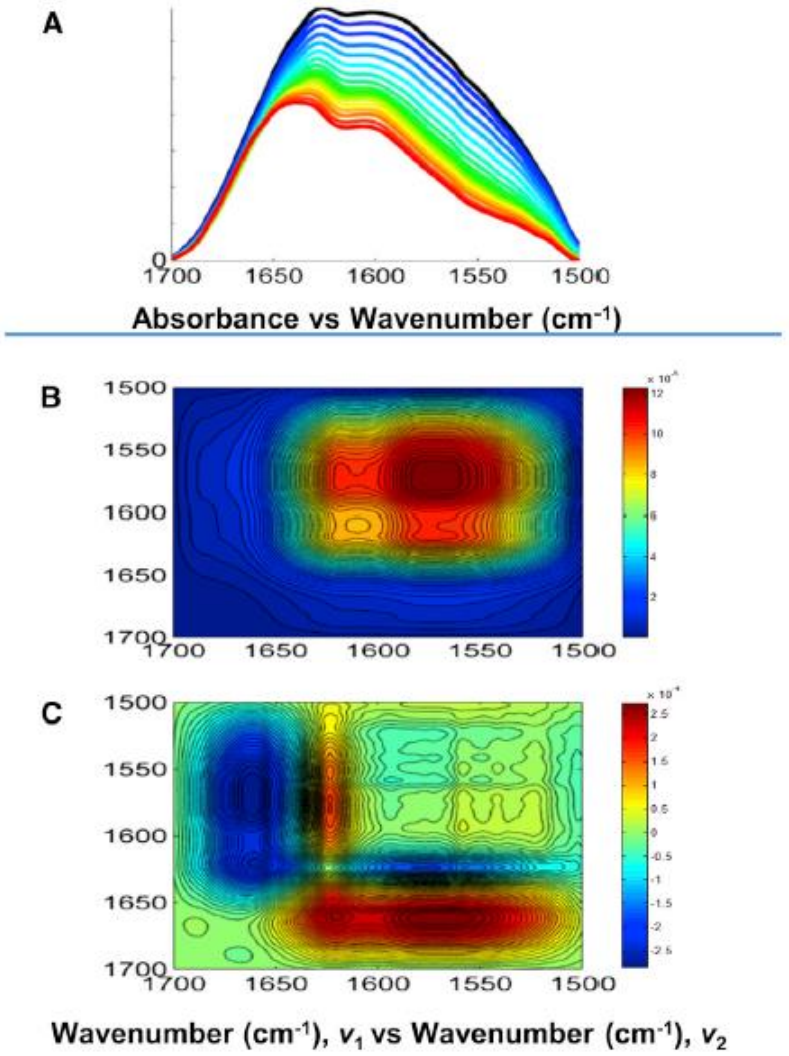

FIGURE 4 2D IR correlation spectroscopy of the ^{13}C-*Cr*cen-*Hs*Prp40Ap complex (1:1 molar ratio). (*A*) Overlaid FT-IR spectra and (*B*) synchronous and (*C*) asynchronous plots within the spectral region 1700–1500 cm^{-1} and over the 5–90°C temperature range with 5°C temperature intervals. In (*A*), the black and red lines correspond to 5°C and 90°C, respectively.

Les résultats

- décrire et interpréter les figures

(A éviter: surnombre des tableaux et figures)

- mentionner les limites et les faiblesses de l'instrumentation ou de la démarche de recueil des données
- la démarche adoptée et des ressources mobilisées
- Normalement pas de références à d'autres études → discussion

La discussion et la conclusion

- mise en valeur de tous les résultats trouvés, sinon leur pertinence est questionnable
- d'après les résultats obtenus et "l'état" du domaine scientifique faire ressortir une problématique
- proposer une conclusion d'après la question de depart en expliquant les résultats amenant à cette conclusion
- conclure en recadrant les limites de la recherche ou des informations présentées
- proposer des perspectives de recherche

Bilan de la lecture:

- texte clair, convaincant et surtout intéressant?
- que vous a apporté sa lecture?
- Présence de biais d'analyse ou "oublis" ou affirmation non justifiées?
- Interprétations/données contradictoires?