

NMR investigation of photoswitchable peptides

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“Praktische Probleme der Kernresonanzspektroskopie“

Erlangen, 18/19.01.2010

FEMP

Light is not only the most important source of energy
but also an important stimulus in signals transduction
pathways of many organisms

Light is also a non-invasive tool in biophysical
investigations

We wanted to design a molecule where light can be
used as a tool to disentangle complex signals

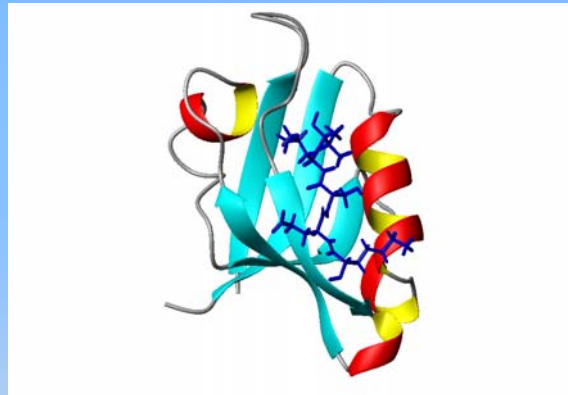
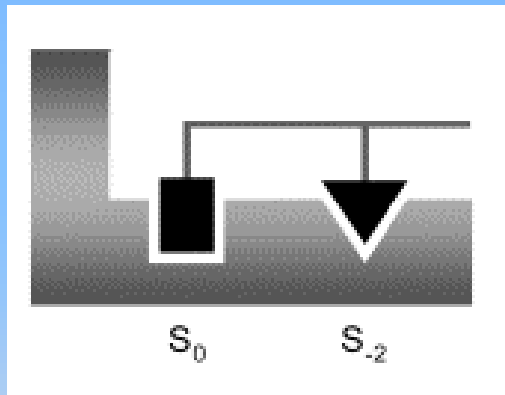
transduction pathways

FEVP

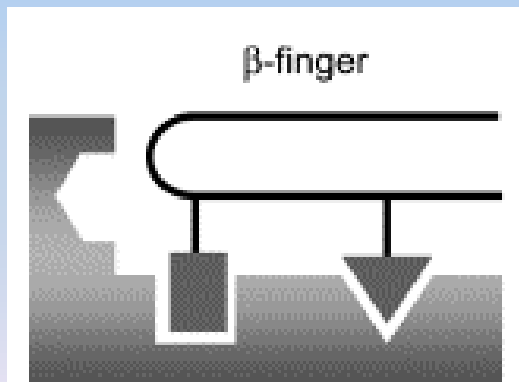
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PDZ-Domains

PDZ domains are protein-protein interaction domains of 100 amino acids



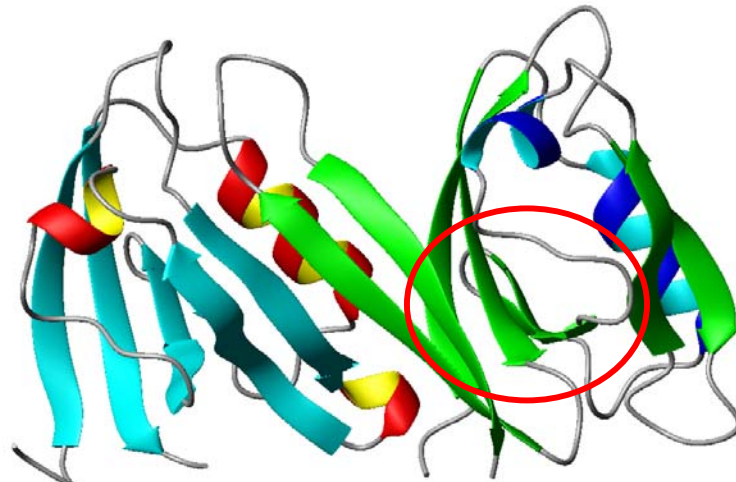
"canonical" binding



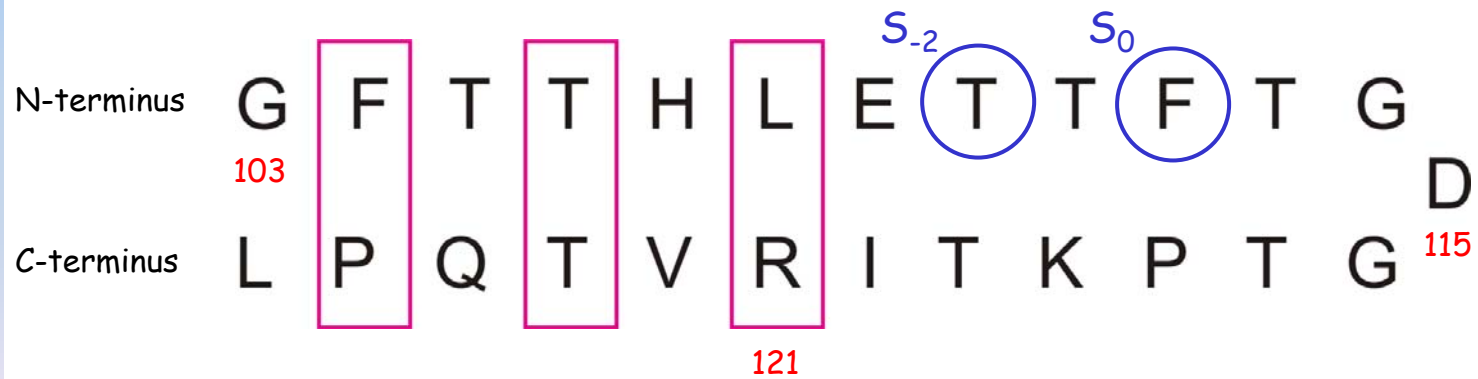
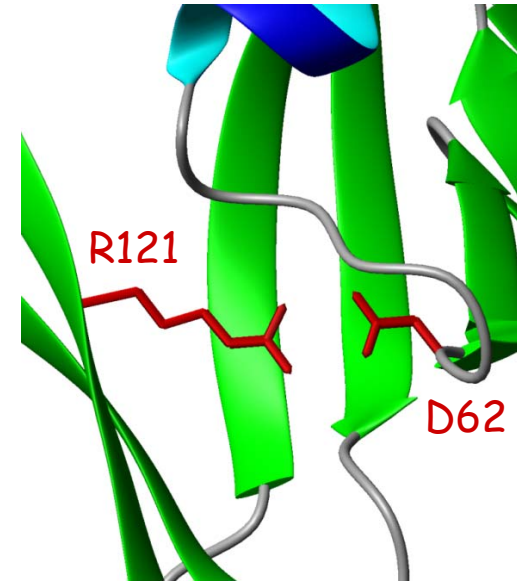
" β -finger" binding

FAVI

Design of the peptides

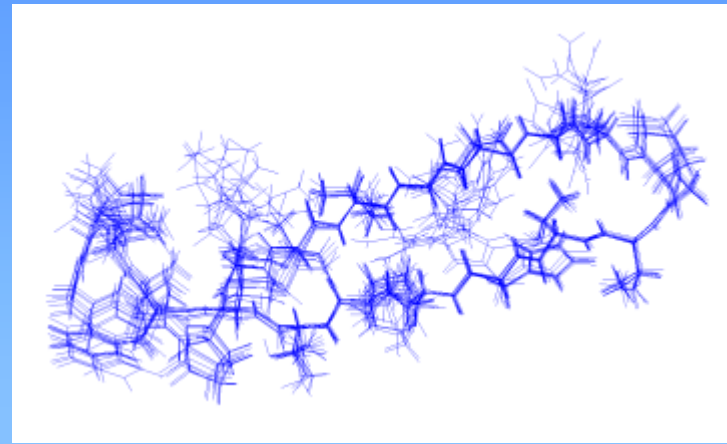


Science 284, 812-815 (1999)

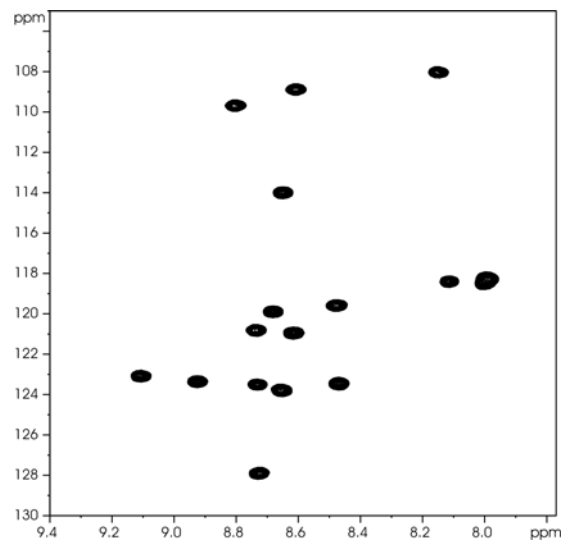


HAUT

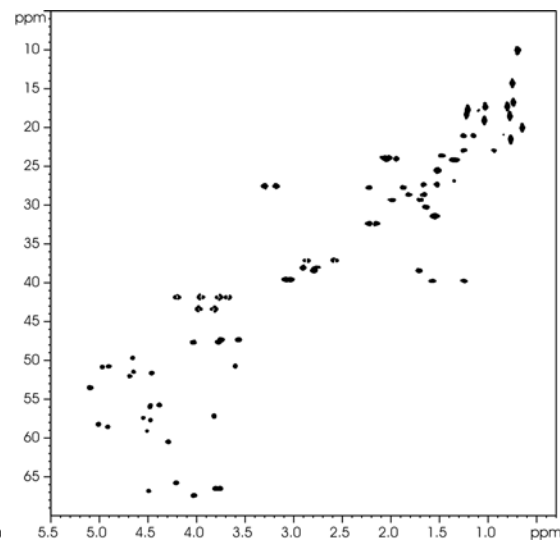
The structure of the peptides was determined using conventional homonuclear techniques but supported by the TALOS approach



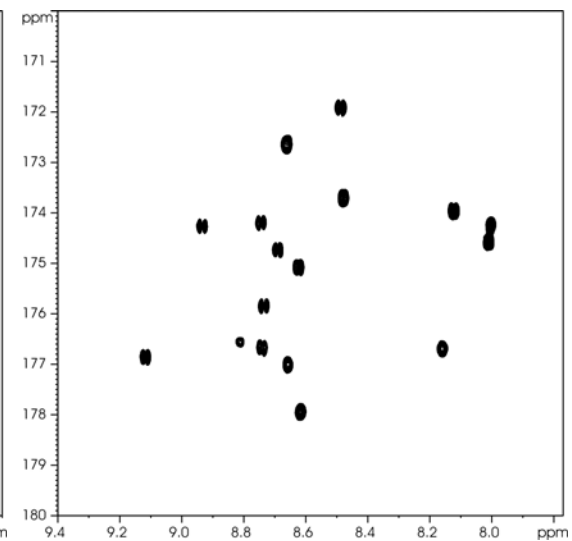
^{15}N -HSQC



^{13}C -HMQC



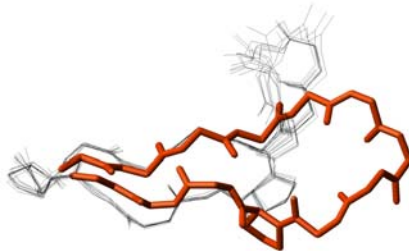
^{13}C -HMBC



FAVT

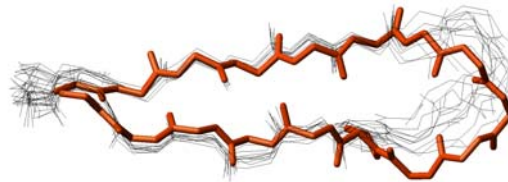
Binding constants

15 aa



$$K_d = 30.4 \pm 5.4 \mu\text{M}$$

19 aa



$$K_d = 3.4 \pm 0.3 \mu\text{M}$$

23 aa



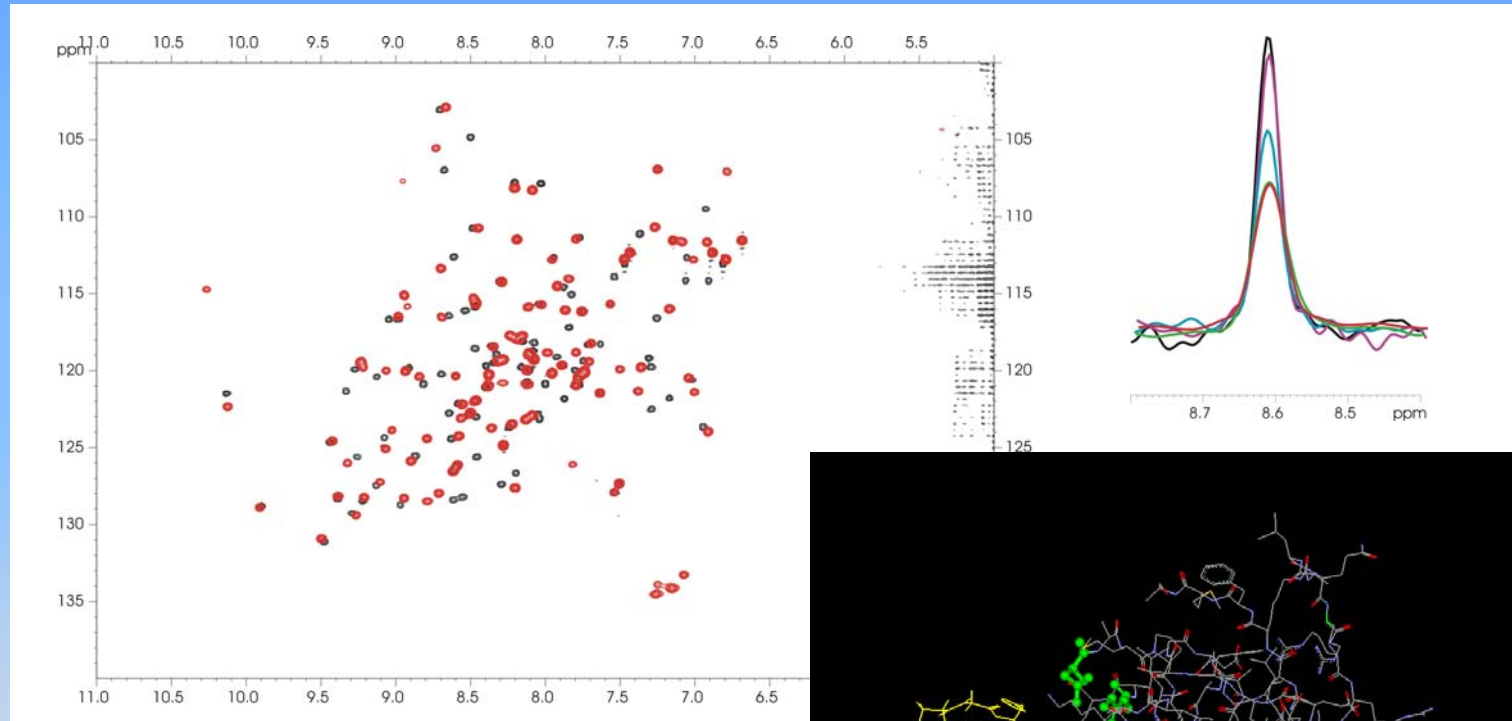
$$K_d = 4.3 \pm 0.1 \mu\text{M}$$

Binding constants were determined by calorimetry (ITC). A linear peptide (VKESLV) gave values similar to those from the literature.

Neither the open chain peptides nor a retro sequence showed detectable binding

FEVMP

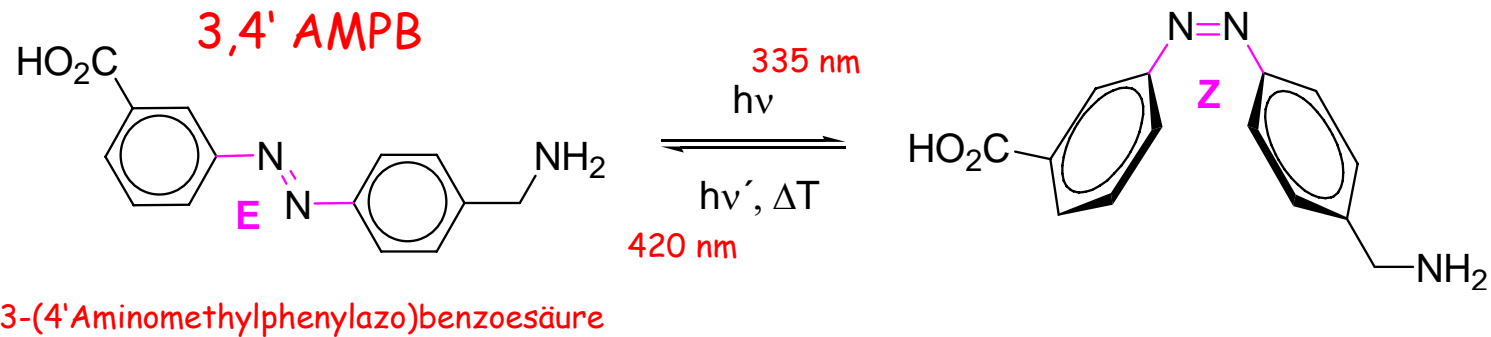
Binding site



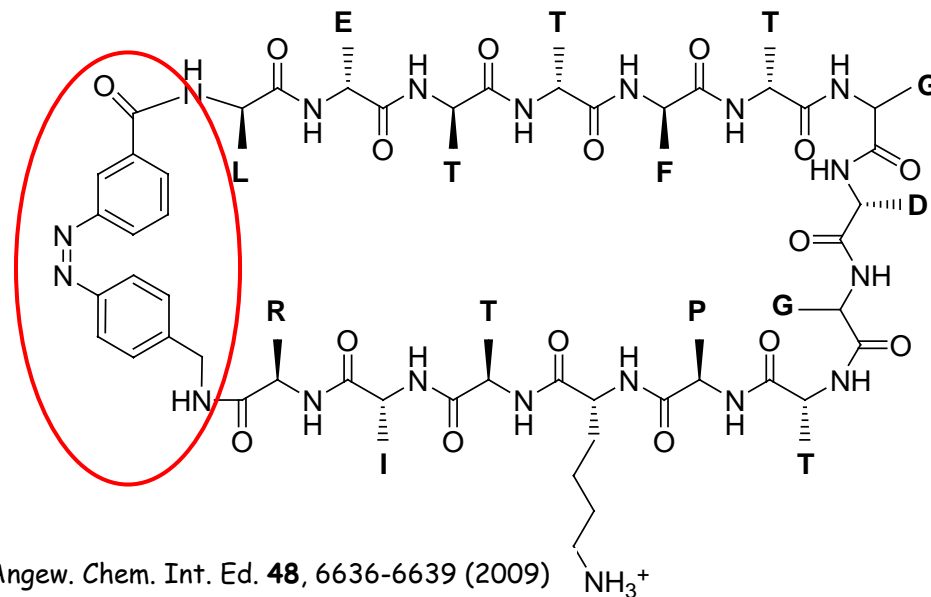
The peptides are
in slow exchange

FEMT

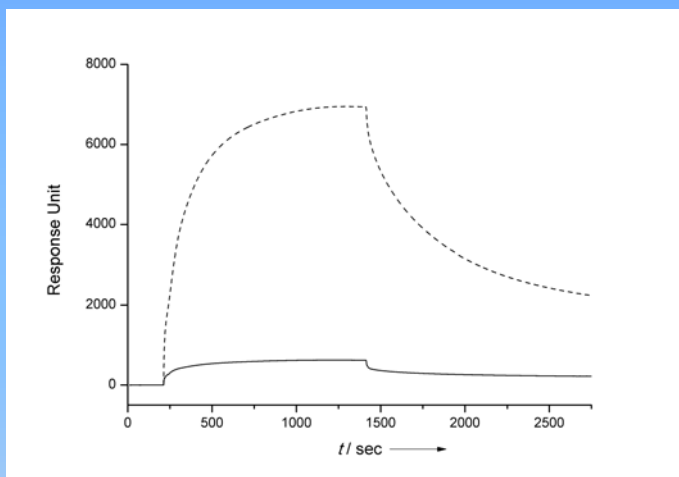
Photoswitchable peptides



The photoswitch
 corresponds to 4 amino
 acids, the peptide has
 "19" amino acids.
trans: no binding
cis: binding

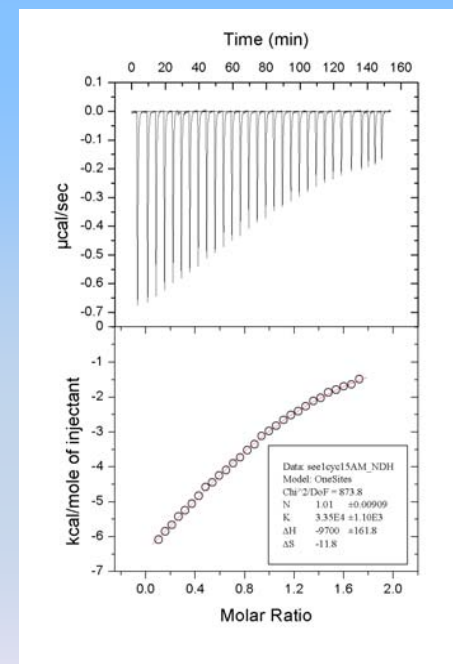
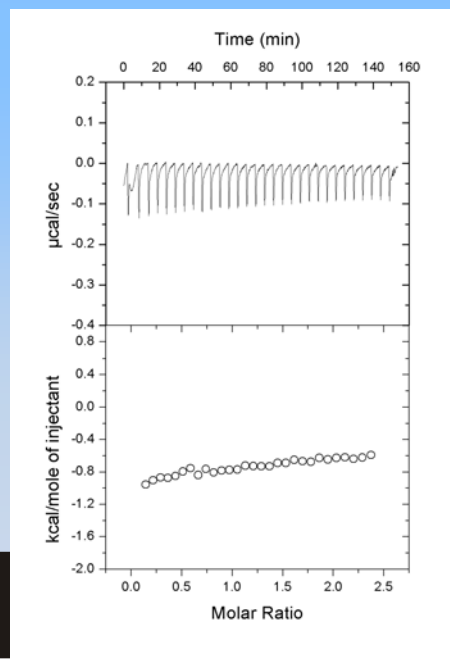


Binding studies (SPR and ITC)



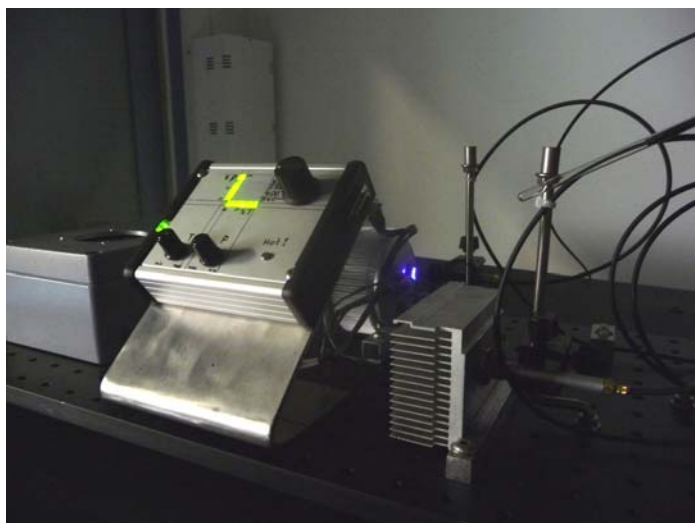
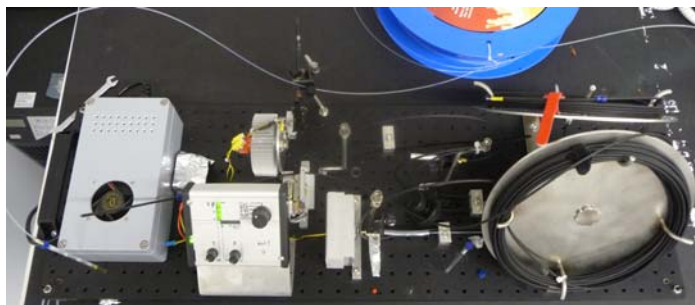
SPR shows clear differences between trans and cis

Using ITC no binding can be detected for trans, but a $K_d = 33 \mu\text{M}$ is determined for cis



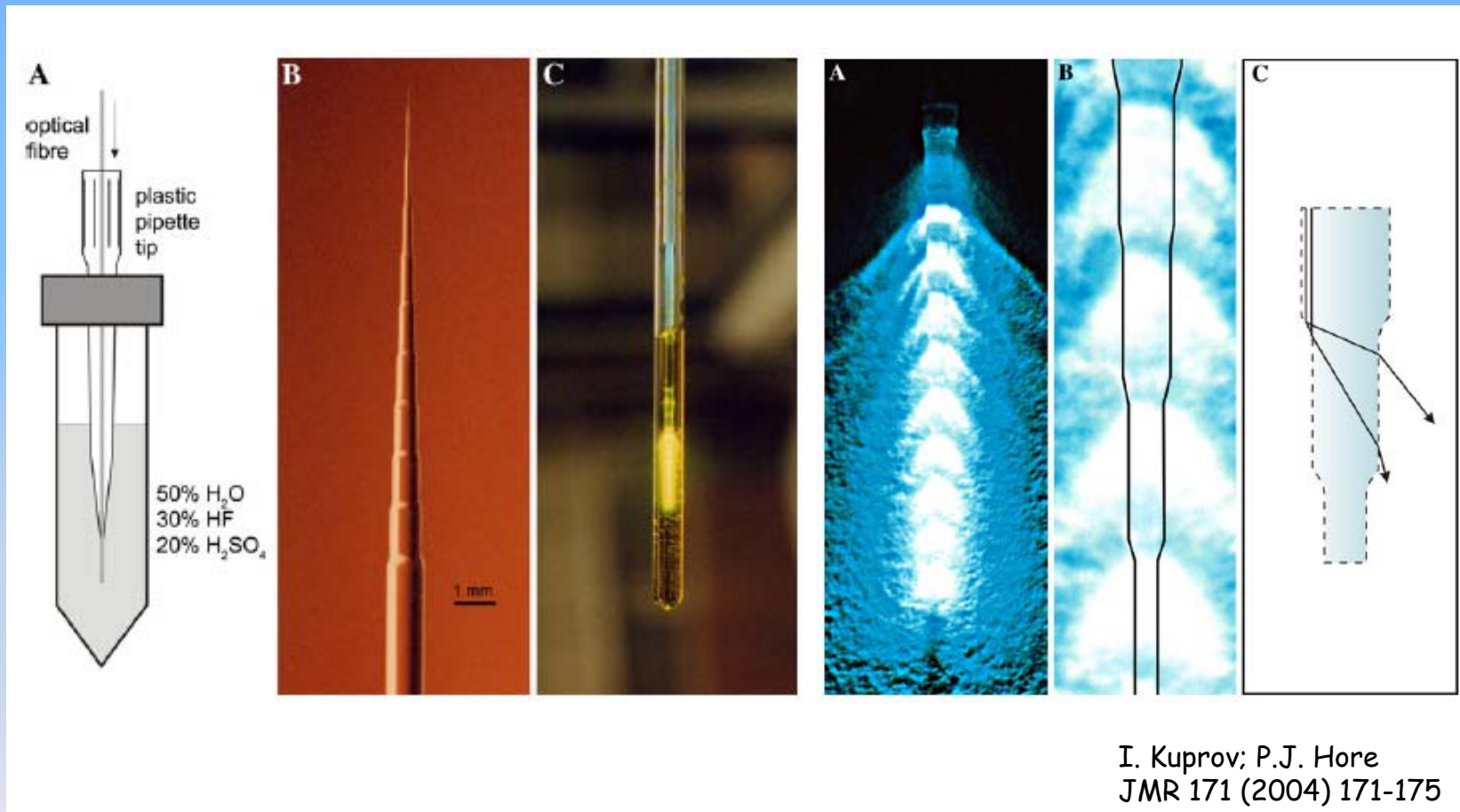
FEVT

Illumination inside the magnet



FEVP

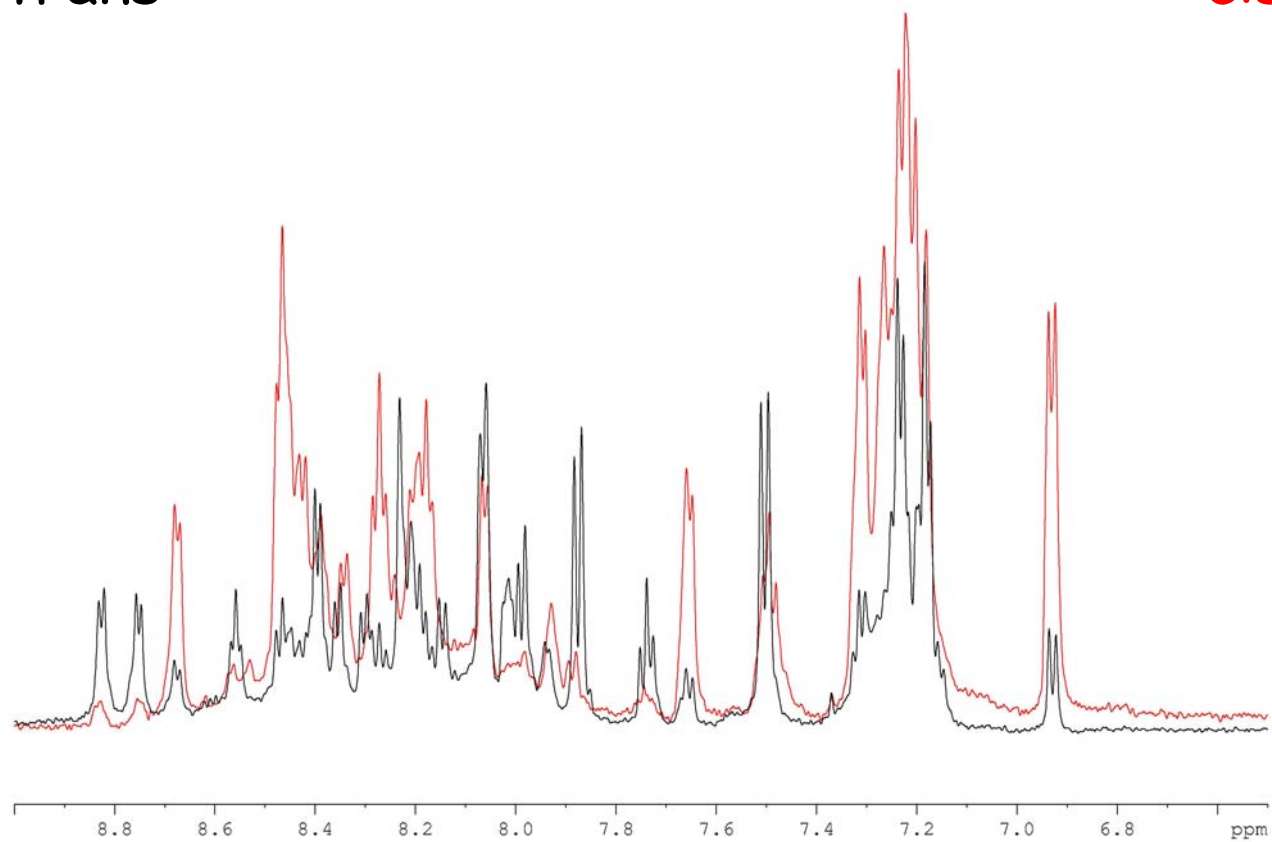
Illumination inside the magnet



NMR with and without light

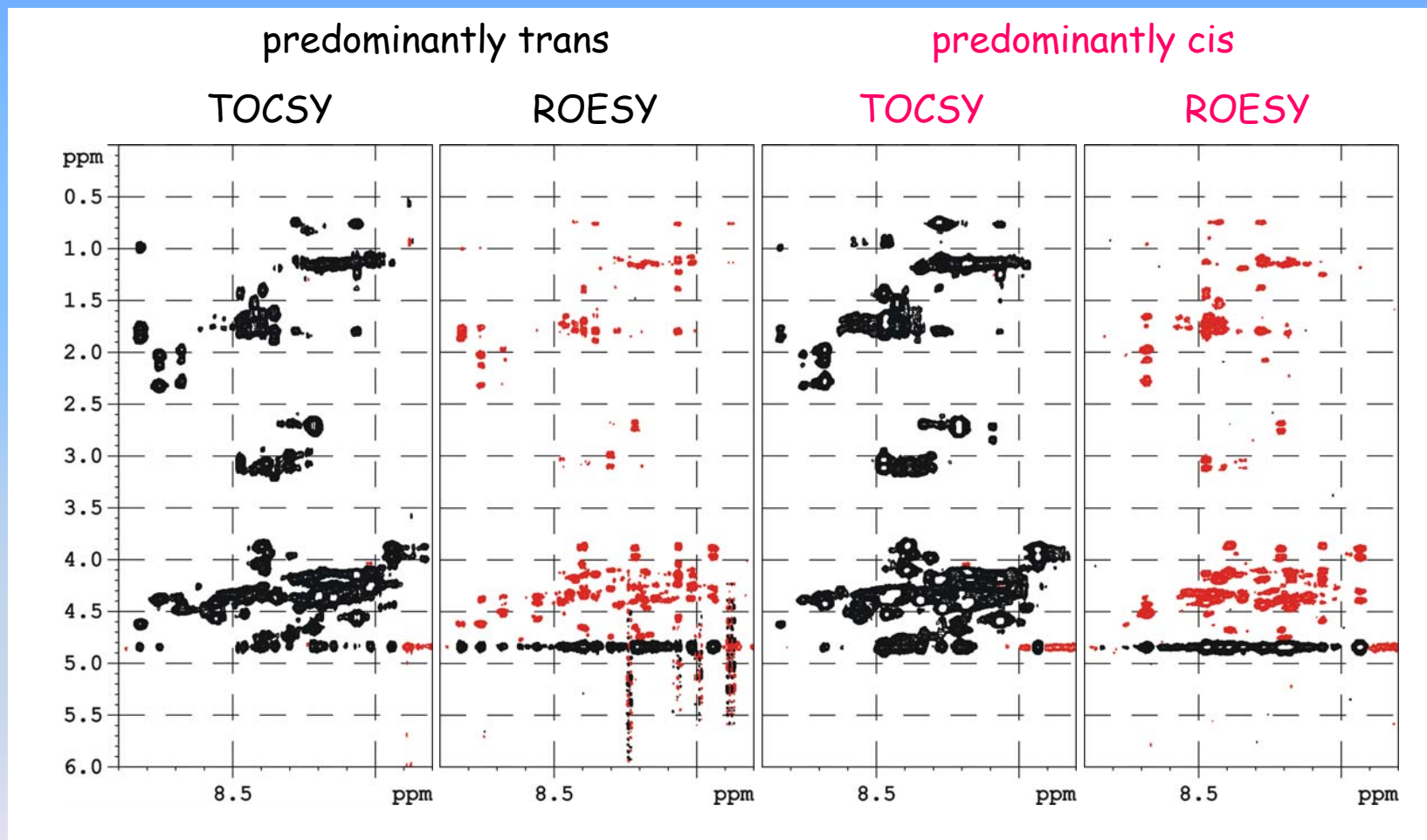
trans

cis



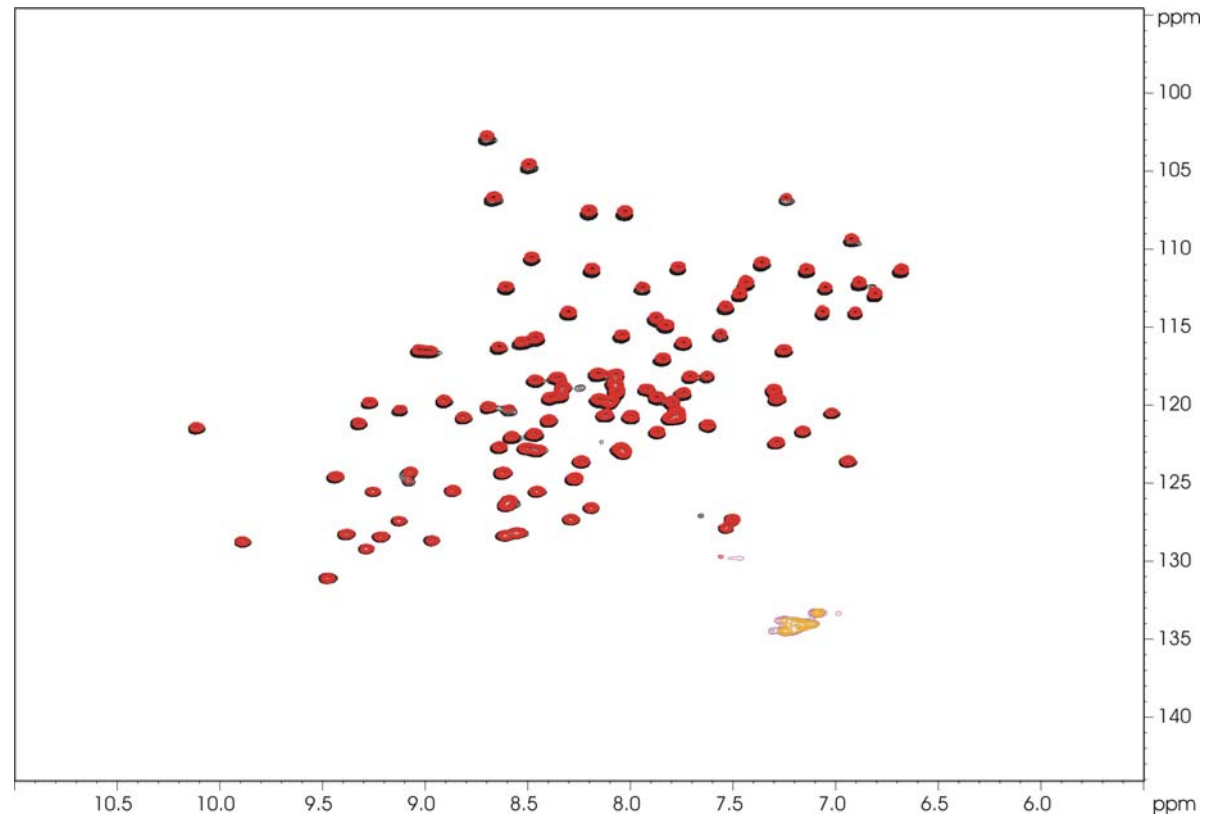
F3VT

NMR with and without light



FAVI

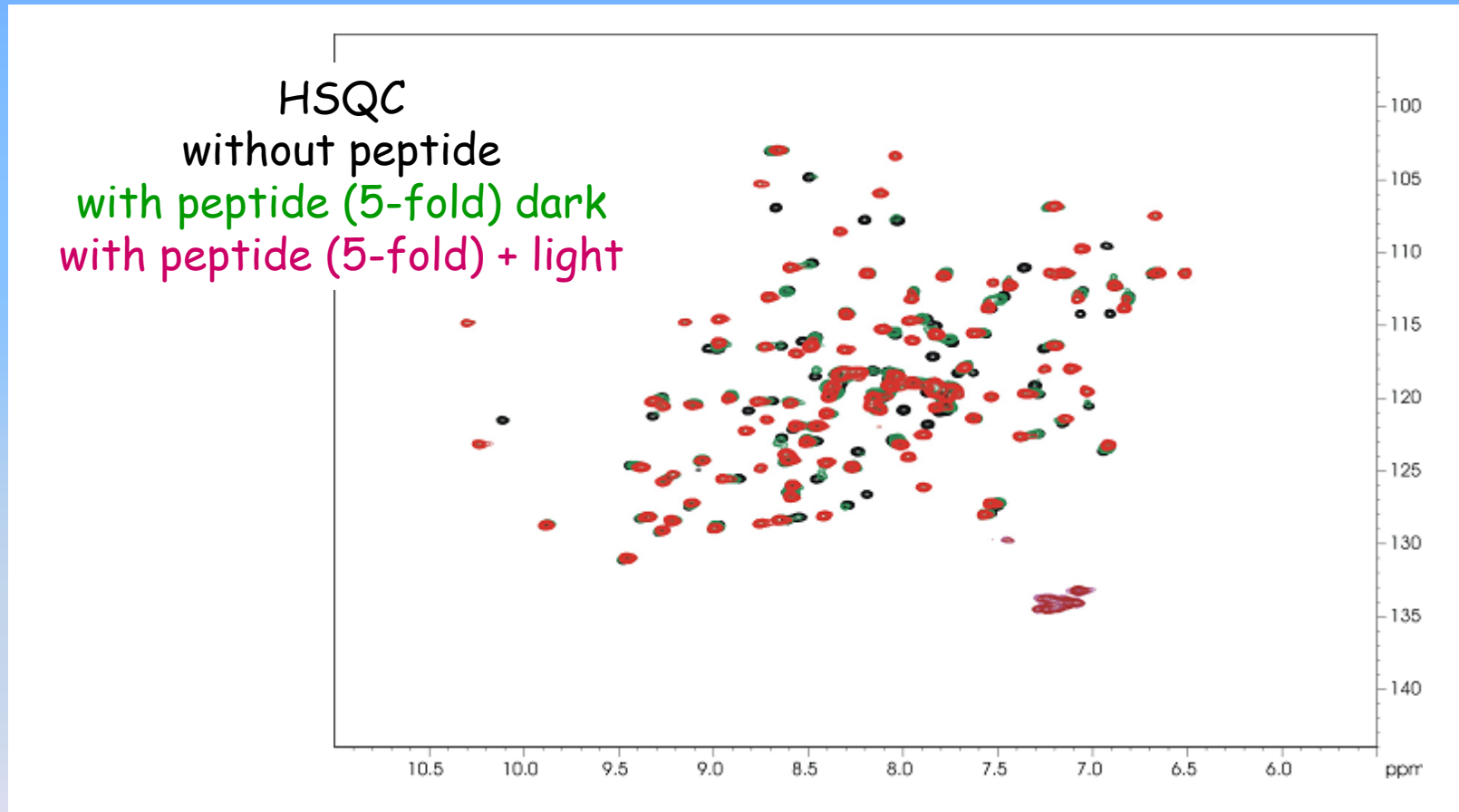
NMR binding studies of the photoswitchable peptide



Continuous irradiation (18h) of the protein alone

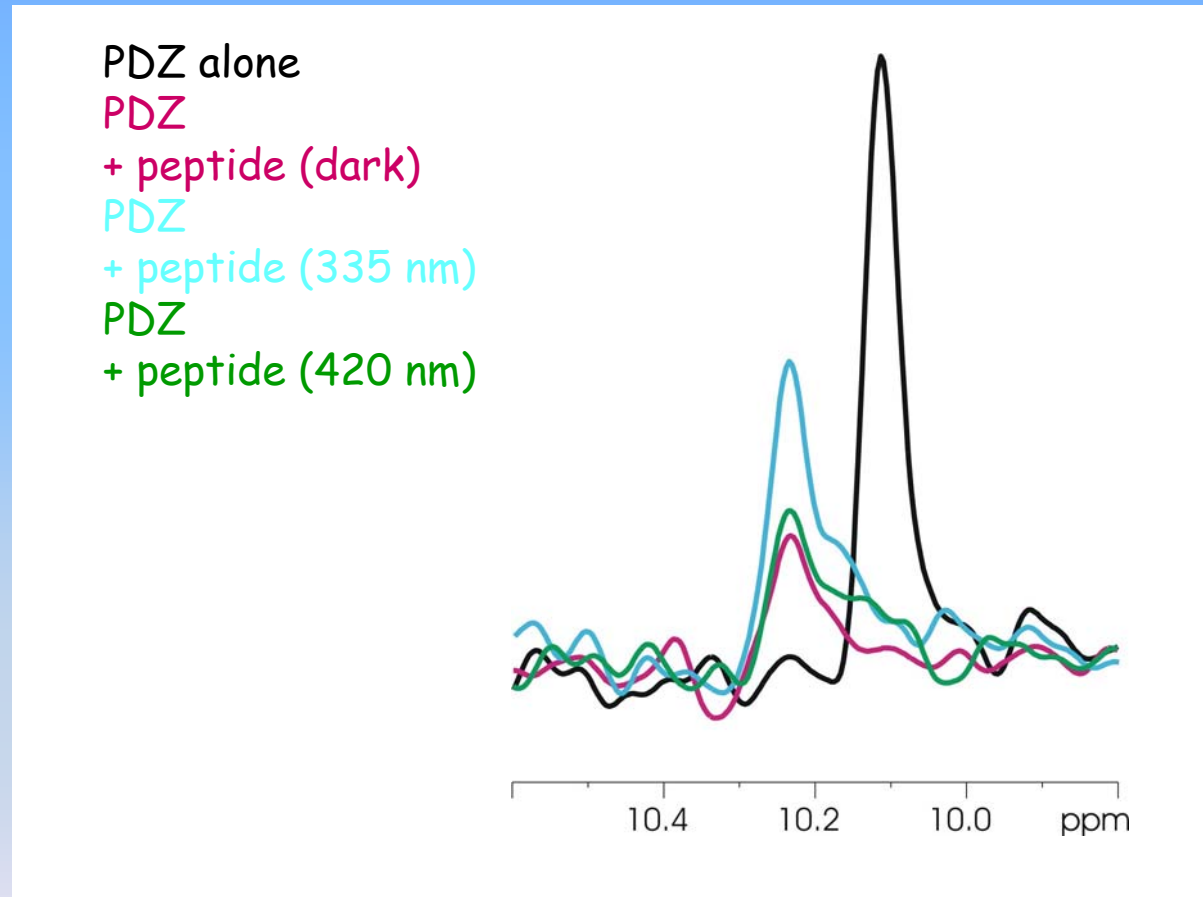
F₂V₂F

NMR binding studies of the photoswitchable peptide



F3VFP

NMR binding studies of the photoswitchable peptide



F₂V₂F

Summary and outlook

A peptide has been designed, synthesized and characterized that can bind to a PDZ domain under the control of light.

The peptide does not have a preformed structure in the binding state.

The peptide still has to prove its usability in a cellular assay

FEMP

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23.01.2010

acknowledgement

FMP

Marco Röben

Sabine Seedorff

Matthias Dorn

Marcel Jurk

Tolga Helmbrecht

Christian Appelt

Monika Beerbaum

Brigitte Schlegel

TU Berlin

A. Richter

K. Rück-Braun

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M. Beyermann

S. Keller

H. Oschkinat

€€€



Bruker

W. Berme

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23.01.2010