Knot tied around an octahedral metal centre

A zinc ion coordinates the folding of a linear polymer programmed to form a knot.

The topology of a molecule has a profound influence on its properties, so a range of sophisticated enzymes has evolved to manipulate molecular entanglements such as knots and chain links. Controlling the topology of synthetic oligomers remains a major challenge, and here we exploit the weak non-covalent interactions in such a molecule to tie a knot around a zinc ion. The folding of the knot is programmed into the covalent structure of the linear molecule, opening new routes for synthesizing macromolecular structures of unprecedented architecture and with well-defined topological properties.

The tools of molecular biology have been used to construct a range of closed knots from DNA and RNA[1,2], and optical tweezers have been applied to tie single DNA or protein molecules manually into open knots[3]. Synthetic strategies for the preparation of small-molecule knots have relied on the templating effects of non-covalent interactions to direct covalent-bond formation[4–7]. Attempts to exploit the ligand geometry around an octahedral metal centre to create a molecular trefoil knot[8] in conventional template synthesis have not been successful. We tested an alternative approach in which a metal ion is added to a presynthesized oligomer equipped with appropriate recognition sites that should cause spontaneous folding into an open knot[9].

We used molecular modelling to design two appropriate building blocks (Fig. 1a), a bidentate ligand (**1**) and a complementary rigid linker (**2**), which we covalently connected to give the linear oligomer **3**. Addition of one equivalent of zinc perchlorate, $Zn(ClO_4)_2$, to a solution of oligomer **3** in deuterodichloromethane caused spontaneous and quantitative conversion to a new species, which was accompanied by marked changes in the proton nuclear magnetic resonance (NMR) spectrum (Fig. 1b). Large shifts were evident in the aromatic signals and the methylene protons became non-equivalent, which suggests that the flexible chains are locked in a single conformation in the complex.

Fast-atom-bombardment mass spectrometry confirmed that a 1:1 complex had been formed and that no higher-order species were present. An X-ray crystal structure of the Zn**3**$(PF_6)_2$ complex (CCDC access no. 163073) proved that the open-knot architecture had been formed in solution. The non-covalent interactions that control folding are apparent in the structure shown in Fig. 1c: metal-ion coordination

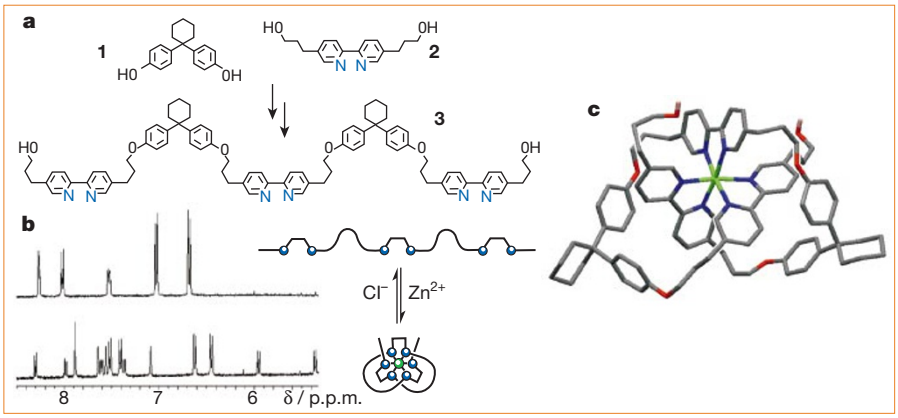

Figure 1 A synthetic oligomer that folds into a knot in the presence of zinc ions. **a,** Building blocks **1** and **2** were used to assemble oligomer **3**. **b,** Reversible folding of the knot complex. Part of the 400-MHz ^{1}H NMR spectrum of **3** in deuterodichloromethane (top) and the corresponding spectrum after addition of one equivalent of $Zn(ClO_4)_2$ (bottom; zinc ion represented in green) are shown. Addition of tetraethylammonium chloride to the zinc complex recovers the spectrum of the unfolded oligomer (top). **c,** Three-dimensional structure of the knot complex, Zn**3**$^{2+}$, as determined by X-ray crystallography (PF_6 anions and CH_3CN solvent molecules are omitted for clarity).

organizes the core of the knot, and the linker bipyridine subunits (**2**) straddle the ligands (**1**) as a result of aromatic interactions that organize the outside of the knot[10].

The folding process is fully reversible. Addition of zinc ions to oligomer **3** quantitatively folds the knot, addition of chloride quantitatively unfolds it to yield the free oligomer, and addition of silver ions (which precipitate the chloride) refolds it. The system can be repeatedly cycled through this process. The open-knot Zn**3**$^{2+}$ represents an important new synthetic intermediate for the assembly of a range of topologically complex molecular architectures. It can be readily prepared on a gram scale, and the terminal hydroxyl groups provide sites for elaboration. Macrocyclization would yield a closed trefoil knot, bulky stopper groups could mechanically trap an open knot, and polymerization would create entangled knotted polymers[11].

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Neurobiology

p25 protein in neurodegeneration

The normal development of the mammalian central nervous system requires a protein kinase known as Cdk5, the activity of which depends on its interaction with a regulatory subunit, p35. An abnormal truncated form of p35 (p25) produced by the action of proteases[1,2] can also activate Cdk5, but causes apoptotic cell death in cultured primary neurons[3]. Patrick *et al.* have reported that p25 accumulates 20–40-fold in brain lysates from patients with Alzheimer's disease, and infer that p25 may contribute to the pathogenesis of neurodegeneration[3]. Here we show that the amount of p25 in the frontal cortex of patients with Alzheimer's disease or Down's syndrome is actually lower than in controls. Although there is evidence for a role for p25 in neurodegeneration from cells in culture[2,3] and in rats[4] and mice[5], our contradictory finding casts doubt on the involvement of p25

in neurodegenerative conditions such as Alzheimer's disease and Down's syndrome.

We tested brains (samples from MRC London Brain Bank for Neurodegenerative Diseases) from seven patients with Down's syndrome (DS; 2 females, 5 males; mean age at death 54.57 ± 7.63 years), six with Alzheimer's (AD; 2 females, 4 males; mean age 61.33 ± 9.42 years) and eight age-matched controls with no neurological or psychiatric history (2 females, 6 males; mean age, 63.50 ± 7.31 years). The principal cause of death was bronchopneumonia for the two sets of patients and heart disease for the controls. Alzheimer samples were verified using standard criteria[6,7]. Post-mortem intervals before dissection were 35.71 ± 19.89, 26.83 ± 22.32 and 37.38 ± 20.51 hours in Down's, Alzheimer's and control brains, respectively.

We compared the levels of p25 in the different brains by probing western blots of brain homogenate (4,000*g* supernatant) with various antibodies: anti-p35 polyclonal C19 (Santa Cruz; 1:2,000), anti-p25 serum (provided by Li-Huei Tsai; 1:1,000), polyclonal N19 antibody against the protease calpain 1 (Santa Cruz; 1:100), monoclonal antibodies against β-actin (Sigma; 1:5,000) or neuron-specific enolase (NSE; Chemicon; 1:5,000).

Figure 1a shows that the intensity of the band containing p25 immunoreactive protein was similar when probed with either anti-p35 polyclonal antibody or anti-p25 antiserum. The amount of immunoreactive p25 was significantly reduced in AD ($P < 0.01$) and DS ($P < 0.05$) homogenates (Fig. 1b, c; left panels). We normalized p25 levels against β-actin and NSE to reveal relative changes in total cells and neuronal cells, respectively. The reduction was comparable in DS frontal cortex when normalized against β-actin, NSE and p35, but only the p25/NSE level was reduced in AD frontal cortex (Fig. 1b, c). In addition, levels of calpain 1 (a protease that produces p25; refs 1, 2) were comparable between groups (results not shown).

Linear-regression analysis reveals no correlation between p25 levels and age, sex, post-mortem intervals or calpain 1 protease concentration in any group (results not shown). Our finding of reduced amounts of p25 in Alzheimer's brains not only disagrees with the results of Patrick *et al.* but raises the possibility that this protein has in fact been downregulated in these patients.

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Patrick et al. *reply* — Yoo and Lubec show that the amount of p25 is decreased in the brains they studied from patients with Alzheimer's disease or Down's syndrome. Their results persuaded us to conduct a more extensive survey of the p25/p35 ratio in AD brains (to be published elsewhere), as the number of samples was small in both of our studies (eight AD brains in our original study and six in theirs). After analysing a further 25 AD brains and those from 25 age-matched controls, we found that p25 levels are consistently higher in AD brains and that the difference is statistically significant (Student's *t*-test). This is in agreement with our original observations[1], as well as being consistent with earlier reports of increased Cdk5 kinase activity in AD brain[2] and of increased amounts of p25 in an animal model of neurodegeneration[3].

Calpain is the protease that processes p35 to generate p25. It has been shown to have increased activity in AD brains[4], but it can also be activated by hypoxic stress. p25 may therefore also be produced post mortem, which would complicate attempts to analyse human brain samples quantitatively. We found that the difference in the amounts of p25 and p35 is most marked in samples with post-mortem intervals of less than 3 hours and decreases in samples with post-mortem intervals of longer than 24 hours. These intervals average 33.3 ± 20.8 hours in the samples studied by Yoo and Lubec, which may explain the high p25 levels in their control samples.

How then can the p25 generated in AD be distinguished from p25 produced post mortem? In our latest analysis, we found p25 from AD brain to be less soluble than that from control brains. This is not surprising, given that p25 and Cdk5 are both increased in neurons containing neurofibrillary tangles. Accordingly, p25 in AD neurons is localized to less soluble compartments, unlike p25 that is generated nonspecifically during the post-mortem period.

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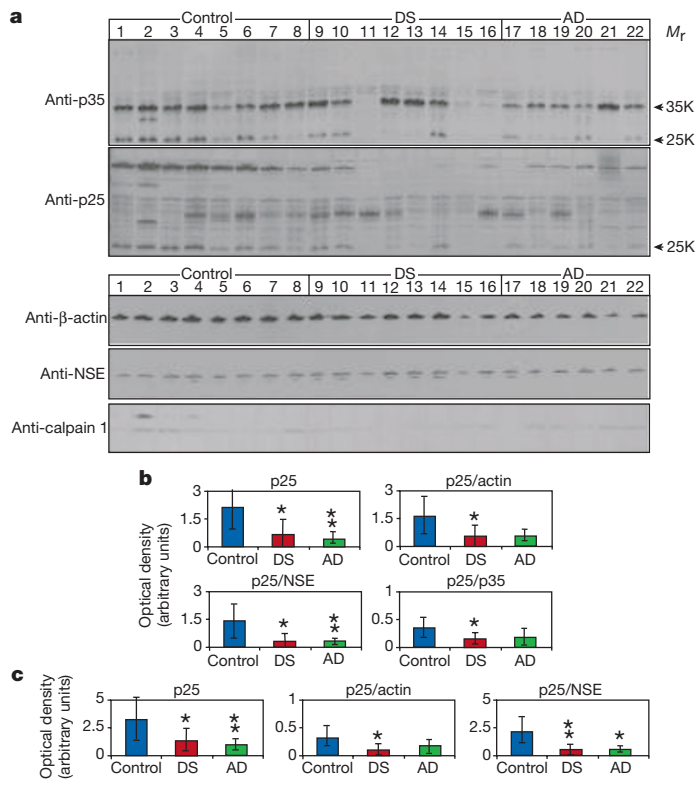

Figure 1 Reduction in the amount of p25 protein in the frontal cortex of brains from patients with Alzheimer's disease (AD) or Down's syndrome (DS). **a,** Western blots of homogenates from the frontal cortex of controls (lanes 1–8), DS (lanes 9–16; lane 15 is a fetal DS sample and data were not used for statistical analysis) and AD (lanes 17–22) probed with antibodies against p35, p25, β-actin, NSE or calpain 1. **b,** Band intensities determined by densitometry of the blots in **a** probed with anti-p35 polyclonal antibody. Levels are shown for p25 and for p25 normalized to actin, NSE or p35 intensities. All protein values are expressed as mean ± standard deviation. Between-group differences were calculated by non-parametric Mann–Whitney *U*-test; statistical significance was set at $P < 0.05$ (single and double asterisks represent $P < 0.05$ and $P < 0.01$, respectively). **c,** As **b**, but using anti-p25 serum. NSE, neuron-specific enolase.