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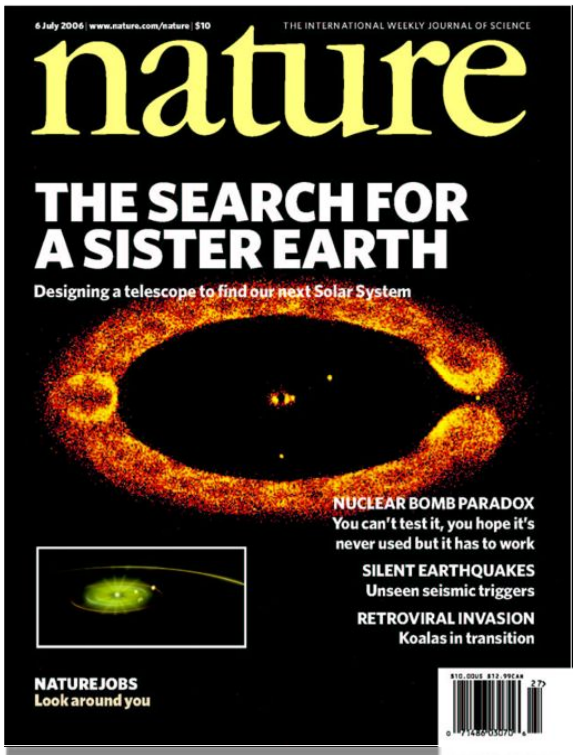

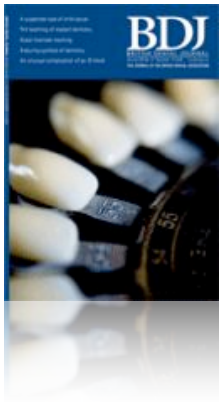
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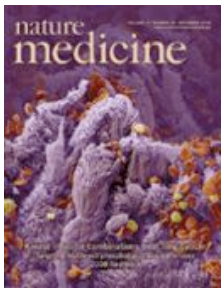
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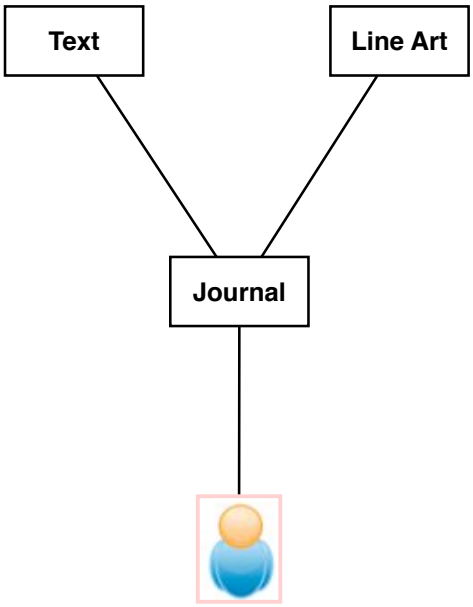

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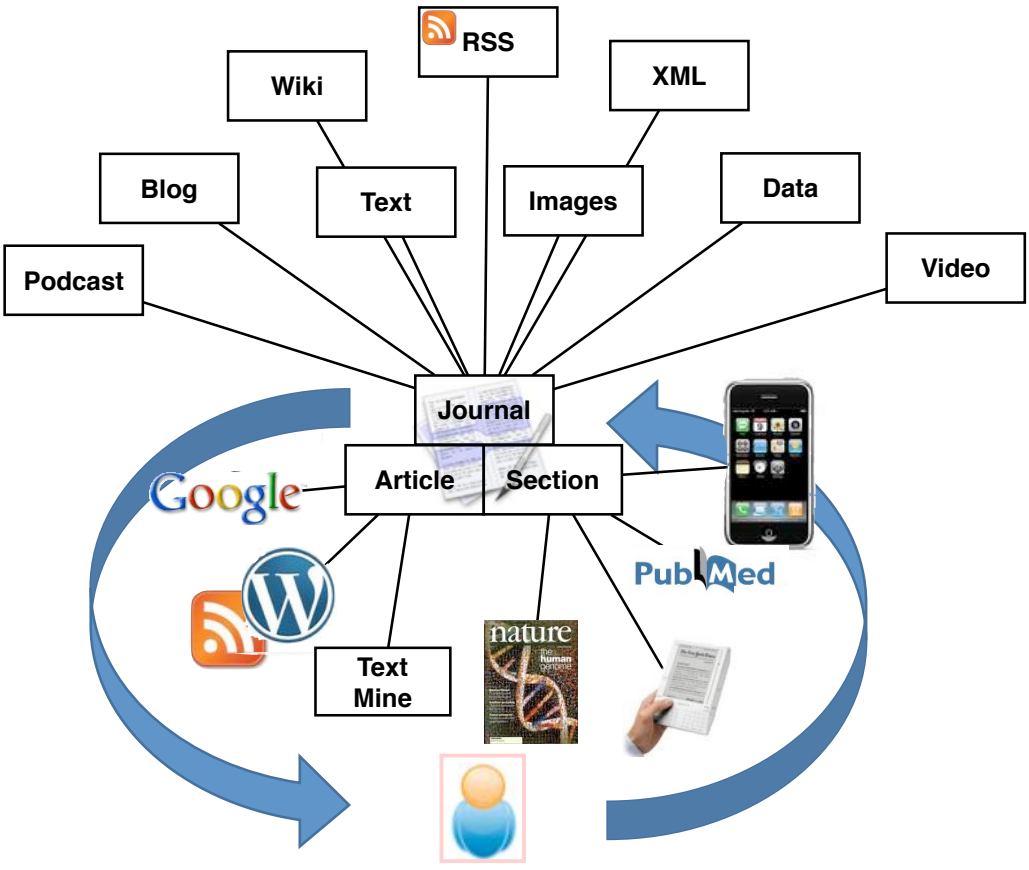

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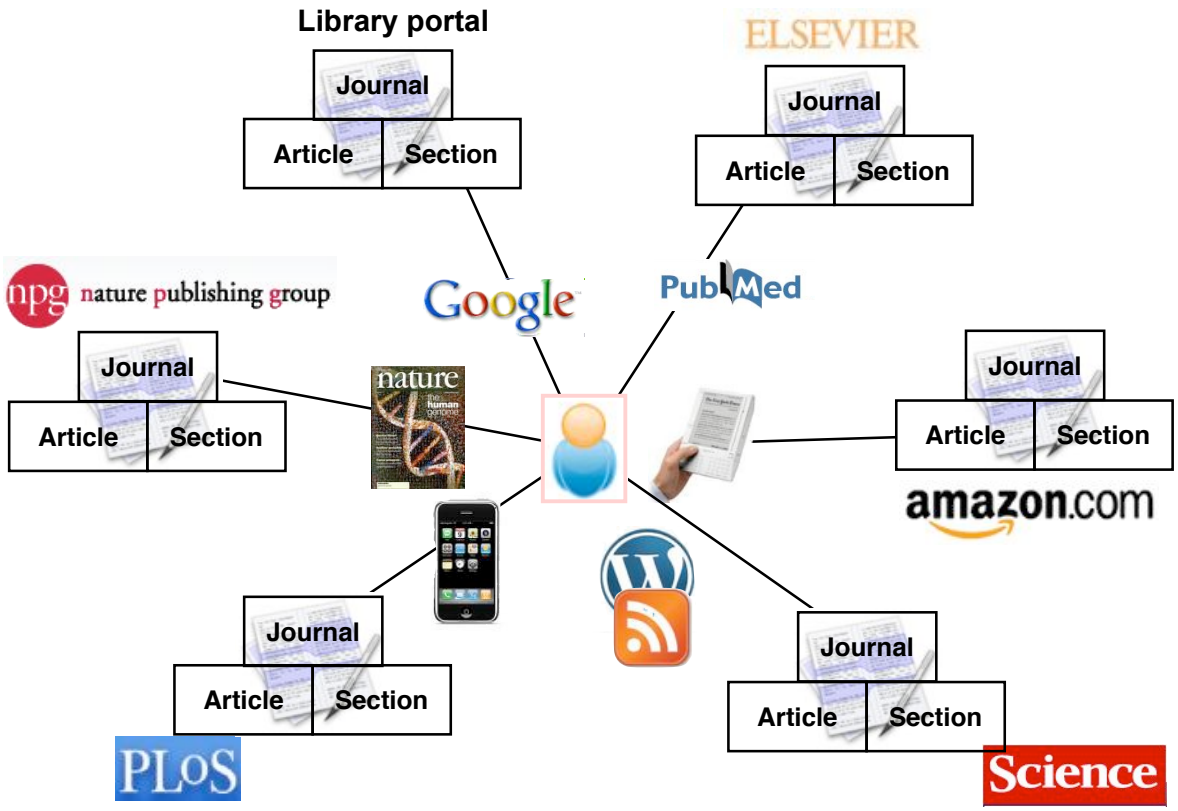

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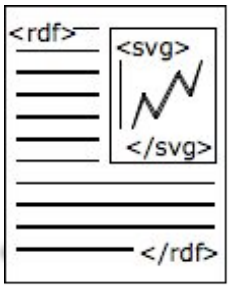

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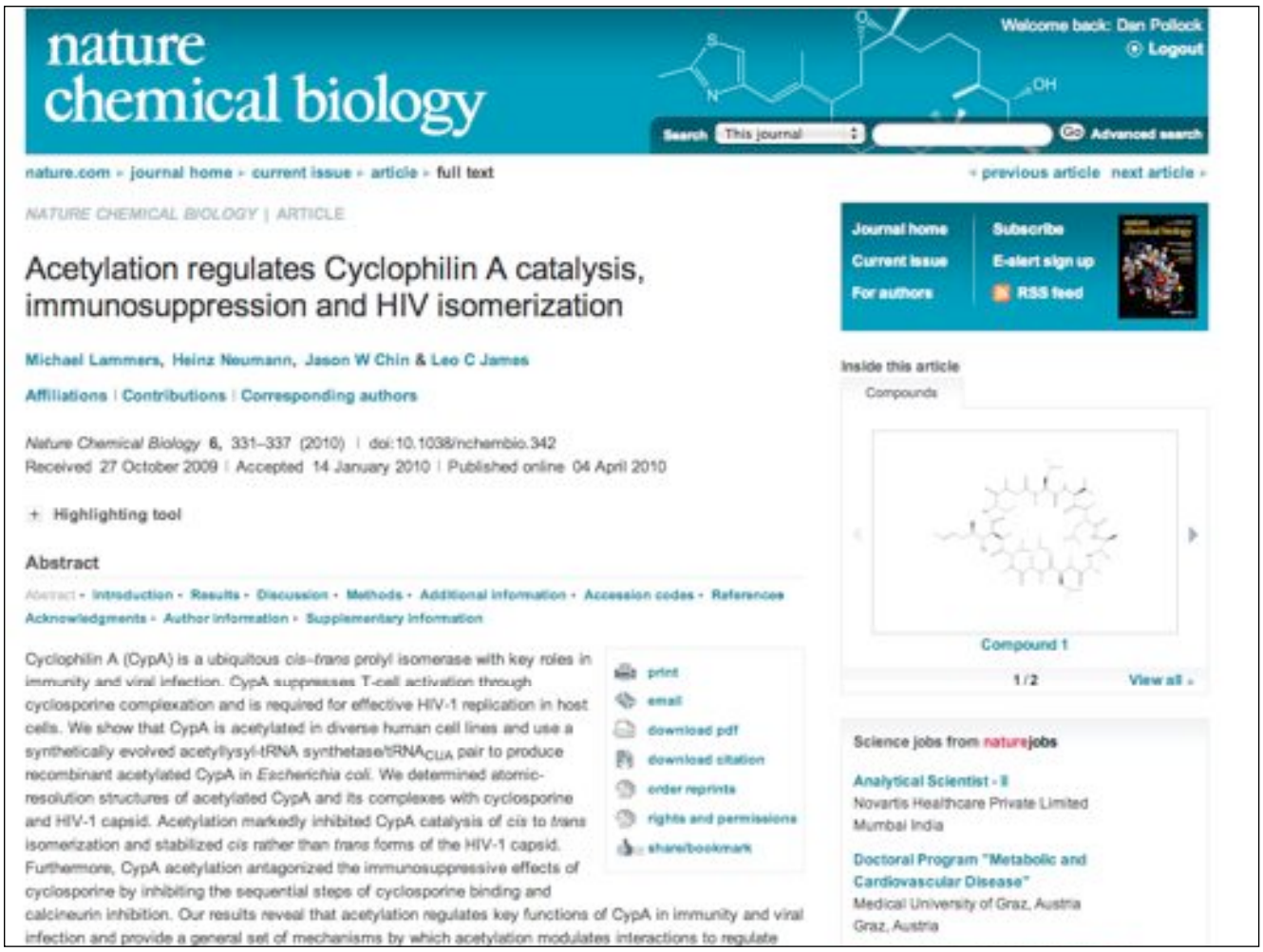
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Cyclophilin A (CypA) is a ubiquitous cis–trans prolyl isomerase with key roles in immunity and viral infection. CypA suppresses T-cell activation through cyclosporine complexation and is required for effective HIV-1 replication in host cells. We show that CypA is acetylated in diverse human cell lines and use a synthetically evolved acetyllysyl-tRNA synthetase/tRNA$_{CUA}$ pair to produce recombinant acetylated CypA in Escherichia coli. We determined atomic-resolution structures of acetylated CypA and its complexes with cyclosporine and HIV-1 capsid. Acetylation markedly inhibited CypA catalysis of cis to trans isomerization and stabilized cis rather than trans forms of the HIV-1 capsid. Furthermore, CypA acetylation antagonized the immunosuppressive effects of cyclosporine by inhibiting the sequential steps of cyclosporine binding and calcineurin inhibition. Our results reveal that acetylation regulates key functions of CypA in immunity and viral infection and provide a general set of mechanisms by which acetylation modulates interactions to regulate
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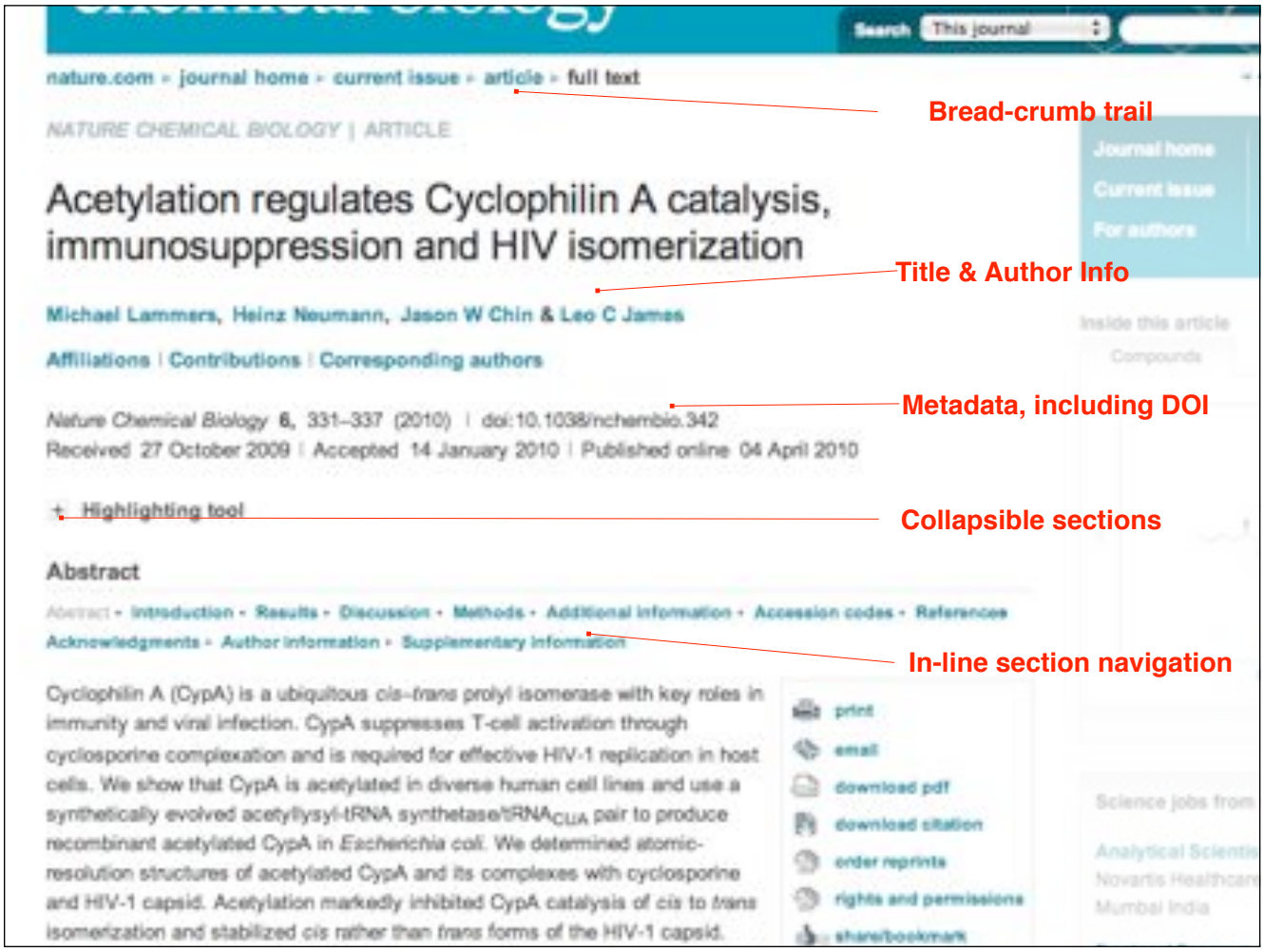
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Abstract

Cyclophilin A (CypA) is a ubiquitous *cis–trans* prolyl isomerase with key roles in immunity and viral infection. CypA suppresses T-cell activation through cyclosporine complexation and is required for effective HIV-1 replication in host cells. We show that CypA is acetylated in diverse human cell lines and use a synthetically evolved acetyllysyl-tRNA synthetase/$tRNA_{CUA}$ pair to produce recombinant acetylated CypA in *Escherichia coli*. We determined atomic-resolution structures of acetylated CypA and its complexes with cyclosporine and HIV-1 capsid. Acetylation markedly inhibited CypA catalysis of *cis* to *trans* isomerization and stabilized *cis* rather than *trans* forms of the HIV-1 capsid. Furthermore, CypA acetylation antagonized the immunosuppressive effects of cyclosporine by inhibiting the sequential steps of cyclosporine binding and calcineurin inhibition. Our results reveal that acetylation regulates key functions of CypA in immunity and viral infection and provide a general set of mechanisms by which acetylation modulates interactions to regulate cell function.

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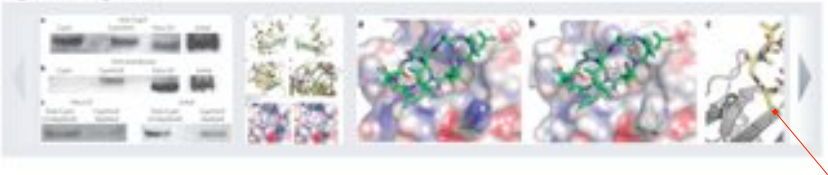

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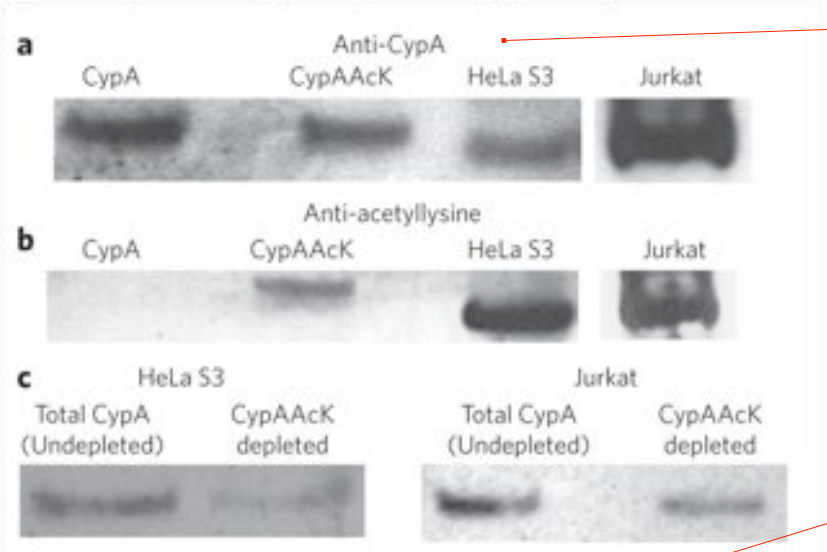
Introduction

Cyclophilins, also known as immunophilins, are peptidyl-prolyl *cis–trans* isomerases found in all kingdoms of life and all tissues. Cyclophilin A (CypA), the archetypal cyclophilin, is central to protein folding[1], signal transduction[2], trafficking[3], receptor assembly[4], cell cycle regulation[5] and stress response and has an expanding catalog of roles[6]. Two of CypA's most important roles in human health are in controlling immunosuppression and viral infection. CypA is the target of the widely used immunosuppressive cyclosporine (1). The complex formed by CypA and cyclosporine binds to and allosterically inhibits calcineurin, a protein phosphatase, leading to suppression of the T-cell mediated immune response by means of an IL-2–mediated downregulation of T-cell activation[7]. CypA is also an essential host protein in the efficient replication of several viruses, including vesicular stomatitis virus[8], severe acute respiratory syndrome

CypA mediates its effects on immunosuppression and HIV-1 infection.

Figure 1: Homogenous and site-specific incorporation of acetyllysine into CypA.



(a,b) CypA can be immunoprecipitated from HeLa and Jurkat T cells (a) and the acetylated fraction detected by anti-acetyllysine in both cell types (b). Recombinant CypA and CypAAcK represent controls. (c) Quantification of acetylated CypA in HeLa and Jurkat cells using an immunodepletion approach. Endogenous CypA from cell lysate was depleted with anti-acetyllysine and compared to undepleted material by western blot with anti-CypA. A substantial proportion of CypA is acetylated in both HeLa and Jurkat cells. See Supplementary Figure 1 for full-length gels and blots.

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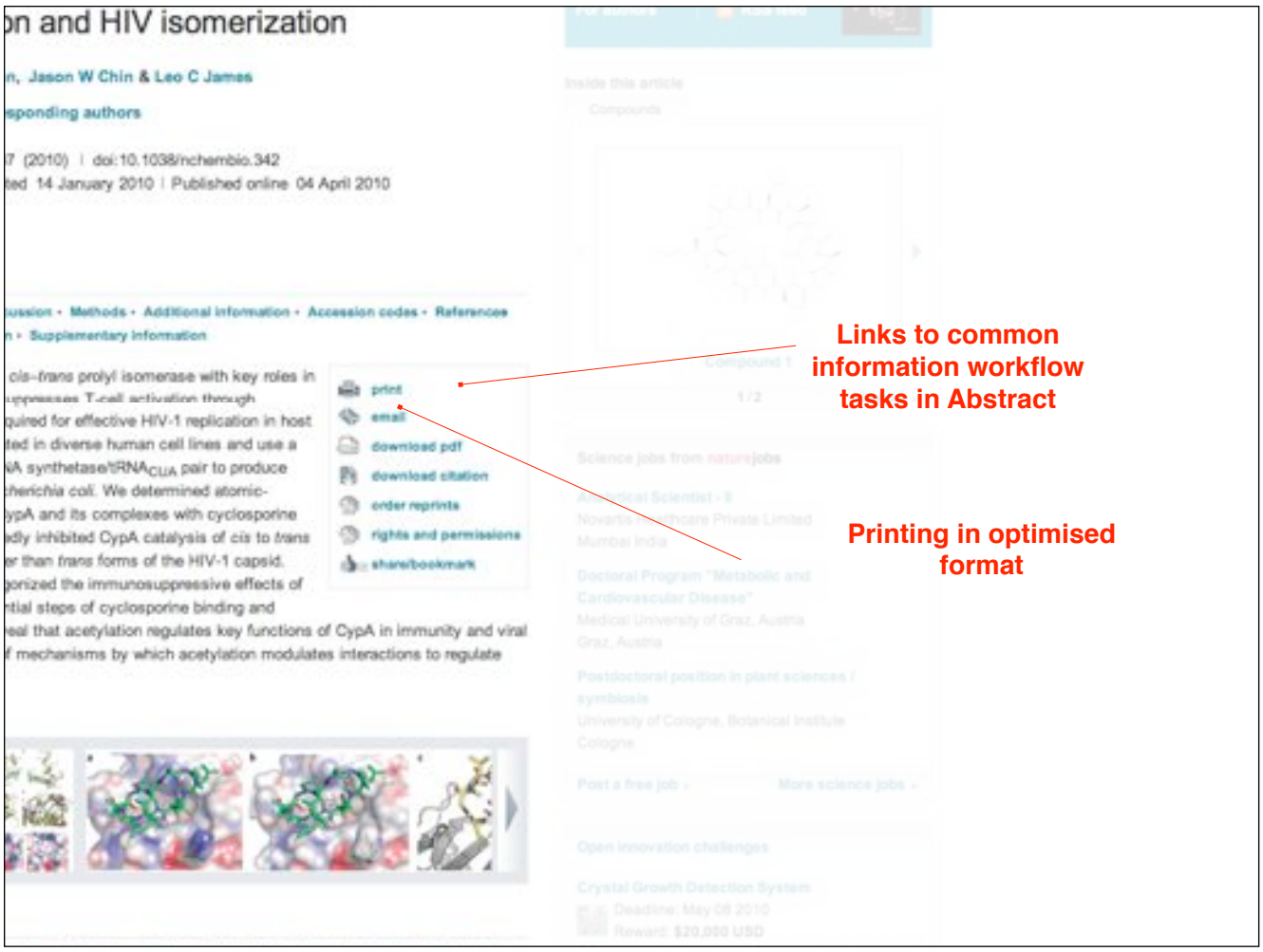
To determine the proportion of endogenous CypA that was acetylated in each cell type, we performed an immunodepletion experiment. Acetylated CypA was depleted from lysate using anti-acetyllysine bound to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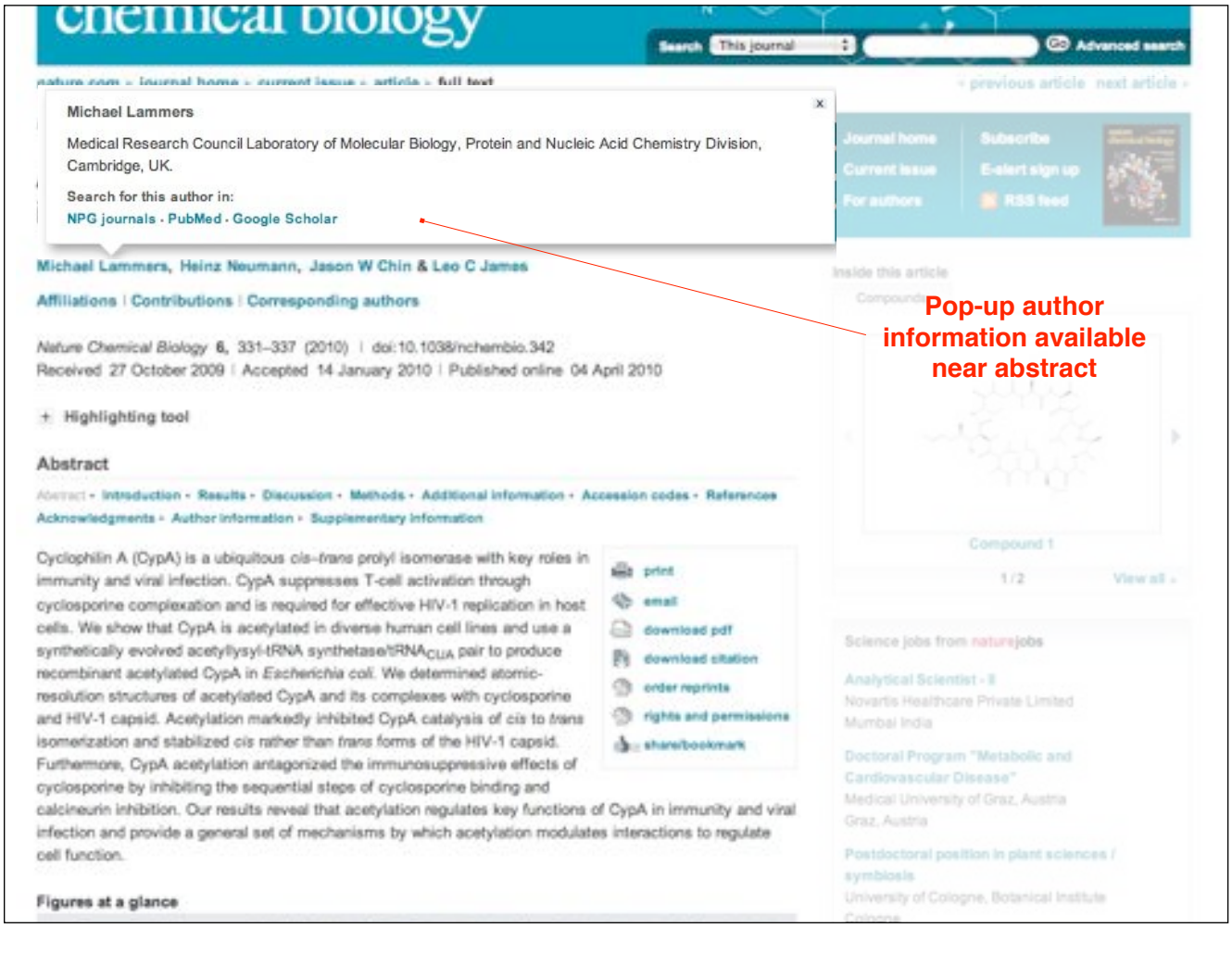
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To determine the proportion of endogenous CypA that was acetylated in each cell type, we performed an immunodepletion experiment. Acetylated CypA was depleted from lysate using anti-acetyllysine bound to protein A–Sepharose. We compared the depleted material, by western blot with anti-CypA, to undepleted material processed in the absence of the anti-acetyllysine (Fig. 1c). To obtain a quantitative estimate of the proportion of acetylated CypA, we performed a standardization experiment with varying ratios of acetylated/unacetylated recombinant protein (Supplementary Fig. 3). Using this standardization, we

+ Acknowledgments

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Contributions

M.L., H.N., J.W.C. and L.C.J. designed experiments and analyzed data.

Competing financial interests

The authors declare no competing financial interests.

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Introduction

Cyclophilins, also known as immunophilins, are peptidyl-prolyl *cis–trans* isomerases found in all kingdoms of life and all tissues. Cyclophilin A (CypA), the archetypal cyclophilin, is central to protein folding[1], signal transduction[2], trafficking[3], receptor assembly[4], cell cycle regulation[5] and stress response and has an expanding catalog of roles[6]. Two of CypA's most important roles in human health are in controlling immunosuppression and viral infection. CypA is the target of the widely used immunosuppressive cyclosporine (1). The complex formed by CypA and cyclosporine binds to and allosterically inhibits calcineurin, a protein phosphatase, leading to suppression of the T-cell mediated immune response by means of an IL-2–mediated downregulation of T-cell activation[7]. CypA is also an essential host protein in the efficient replication of several viruses, including vesicular stomatitis virus[8], severe acute respiratory syndrome (SARS) virus[6], hepatitis C virus (HCV)[8], vaccinia virus and HIV type 1 (refs. 9,10). During HIV-1 infection, CypA interacts with the capsid protein gag and is packaged into budding HIV-1 virions[9, 10]. Inhibition of CypA by cyclosporine substantially decreases viral titer in human cells[11]. The importance of CypA in lentiviral replication is underscored by the independent evolution in two different primate species of antiviral restriction factors that use a retrotransposed copy of CypA to provide viral targeting[12]. Despite the importance of CypA in immunosuppression, viral infection and other key cellular processes, the molecular mechanisms by which the varied and crucial functions of CypA are regulated remains unclear.

Acetylation of the ε-amine of specific lysine residues in proteins is a reversible post-translational modification with diverse roles and a functional importance that rivals that of phosphorylation[13]. Acetylation is mediated by acetyl-CoA–dependent histone acetyltransferases[14] and reversed by zinc-dependent histone deacetylases or NAD dependent sirtuins[15]. Acetylated targets can be specifically recognized by bromodomain-containing proteins[16]. Recent mass spectrometry and immunofluorescence studies demonstrate that hundreds of nonhistone proteins are acetylated in mammalian cells[17]; however, the molecular mechanisms by which acetylation may control protein function and effect cellular regulation are largely unknown.

A recent proteomics screen isolated a peptide whose sequence matched CypA but which contained an N^{ε}-acetyl-L-lysine (acetyllysine, 2) in place of Lys125 (ref. 17). However, because there are many CypA gene fusions in the genome, the origin of this peptide was ambiguous. Here we show that the free enzyme form of CypA is acetylated in human cells. We produced homogeneously and site-specifically acetylated recombinant CypA using an acetyllysyl-tRNA synthetase/$tRNA_{CUA}$ pair that co-translationally directs the incorporation of acetyllysine in response to an amber codon[18] placed in a CypA gene. This approach allowed us to perform

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material processed in the absence of the anti-acetyllysine (Fig. 1c). To obtain a quantitative estimate of the proportion of acetylated CypA, we performed a standardization experiment with varying ratios of acetylated:nonacetylated recombinant protein (Supplementary Fig. 2). Using this standardization, we estimated that 50% ± 5% (s.d.) of CypA was acetylated in HeLa cells and 38% ± 3% in Jurkat T cells. These results indicated that a substantial proportion of CypA was acetylated in both HeLa and Jurkat T cells. Although we expect the proportion of acetylated protein to be regulated in response to stimuli, these results demonstrate that the effects of acetylation on CypA are relevant to, and important for, understanding CypA-

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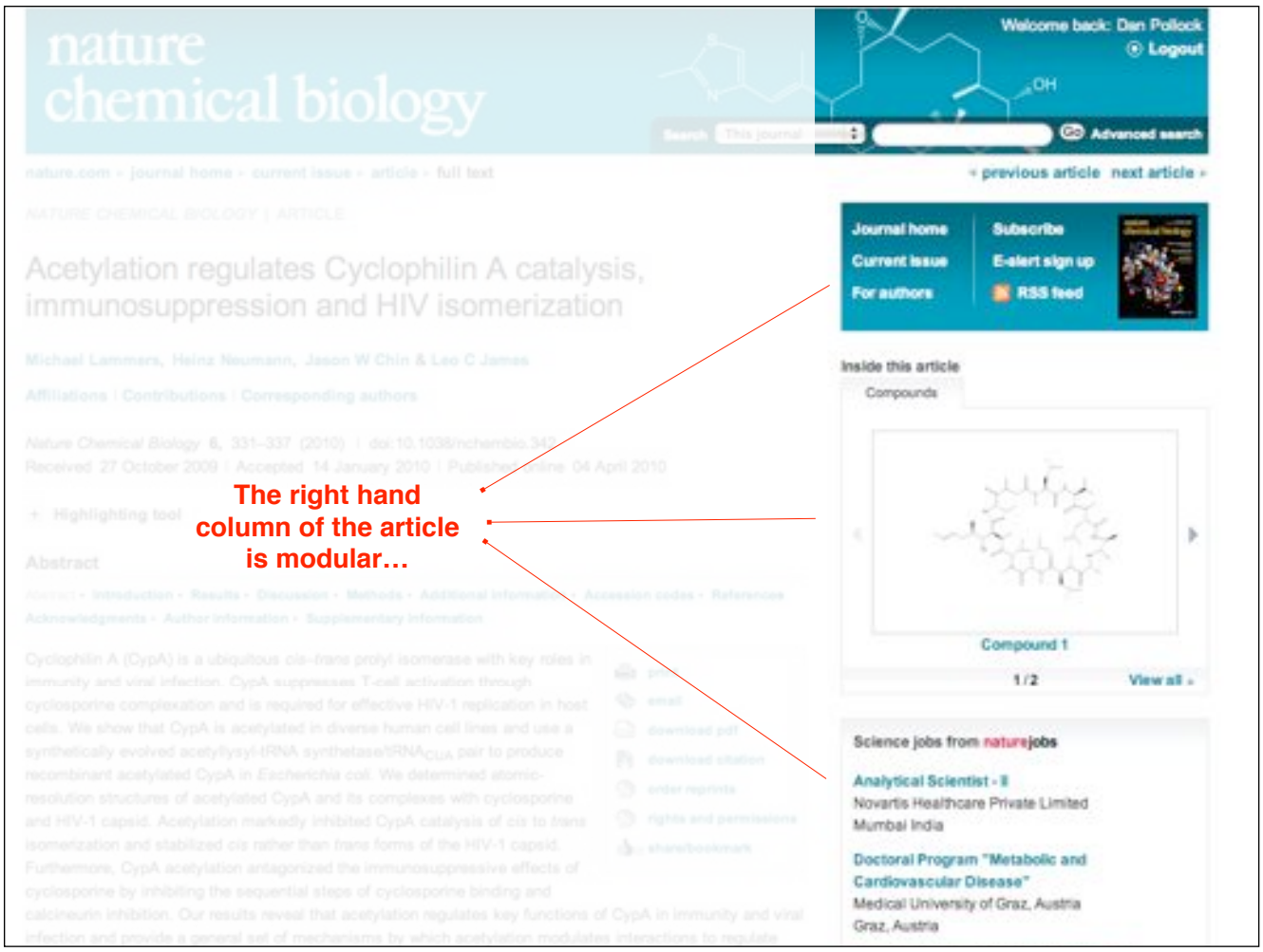
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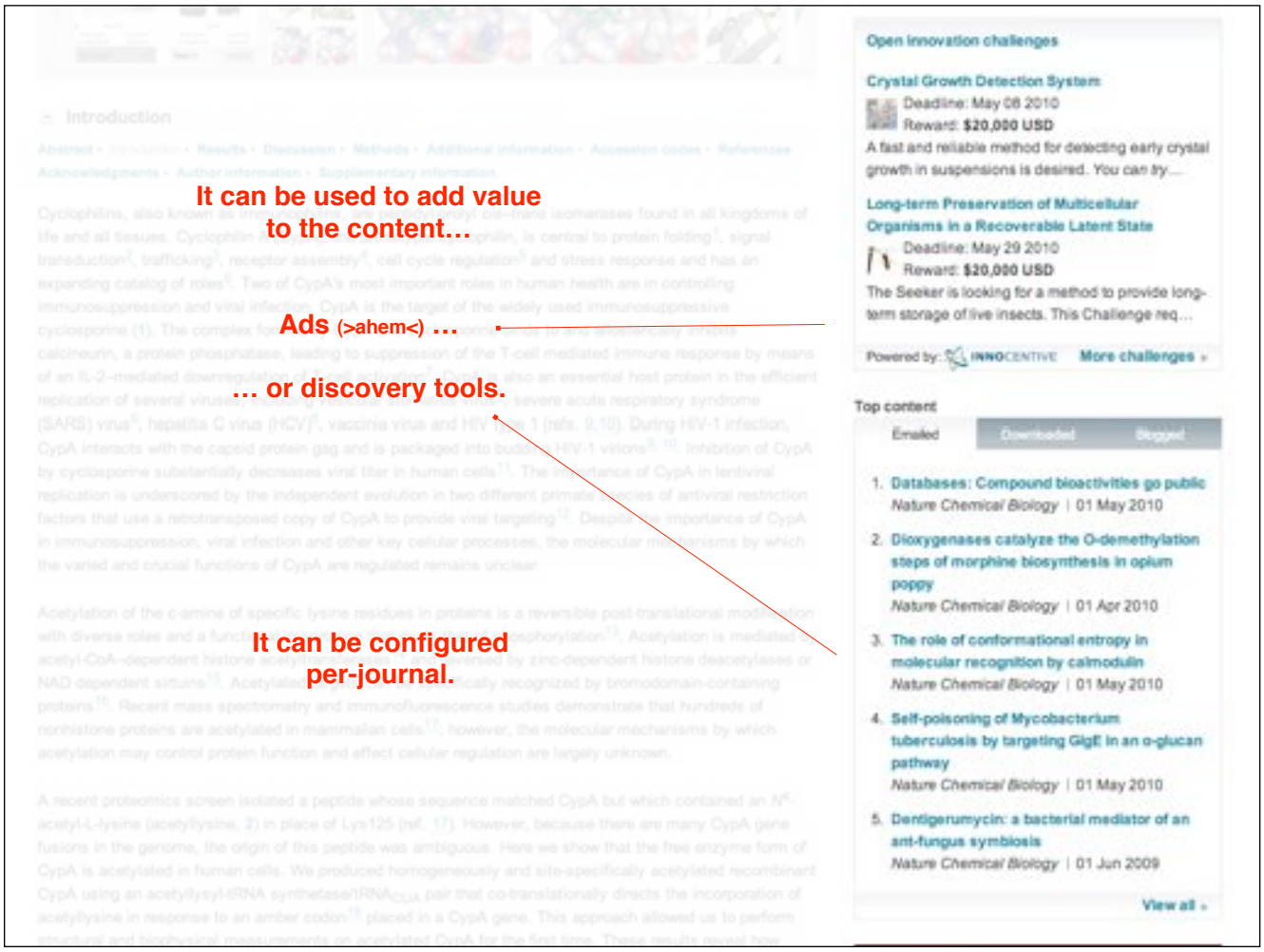
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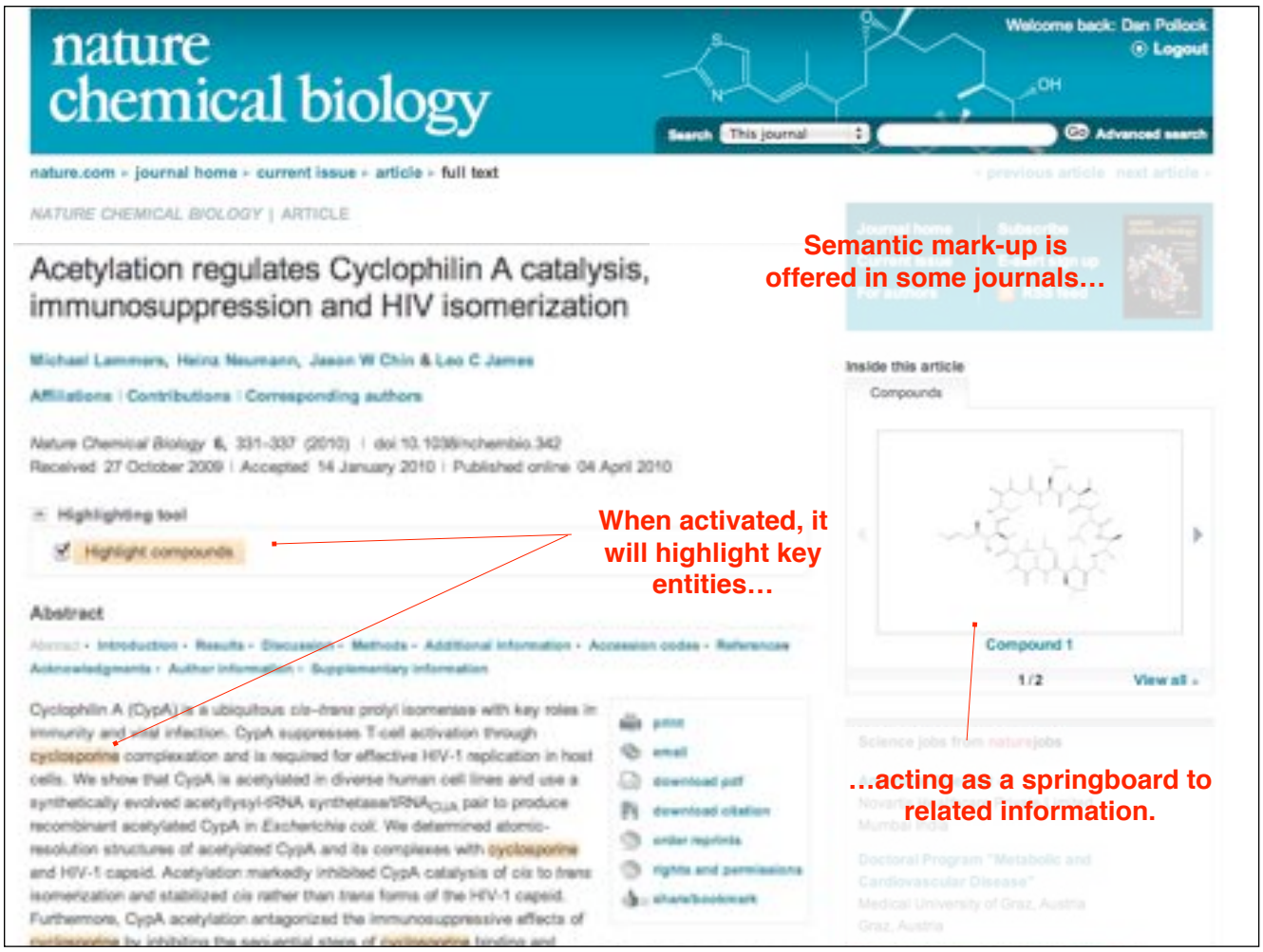
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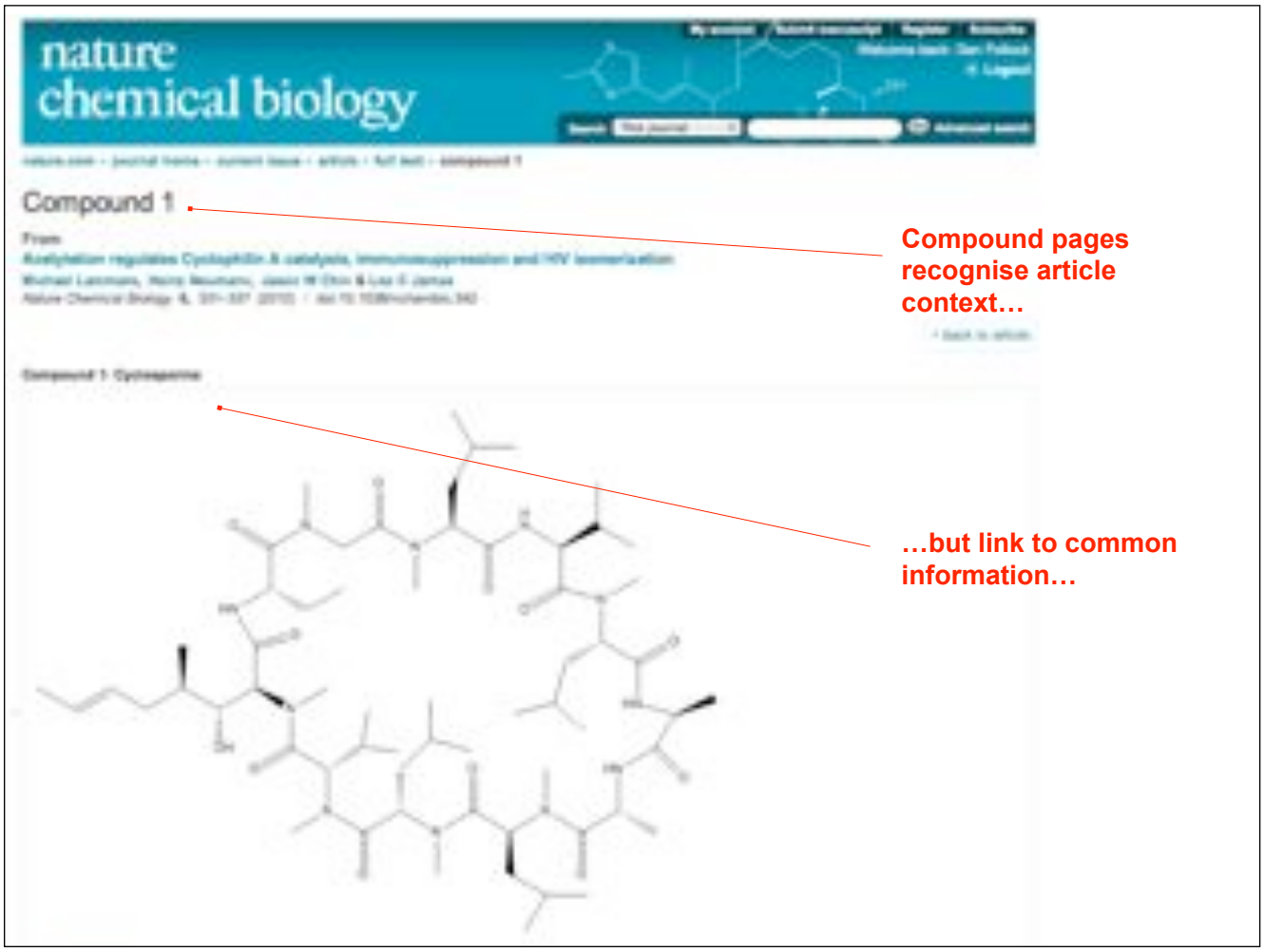
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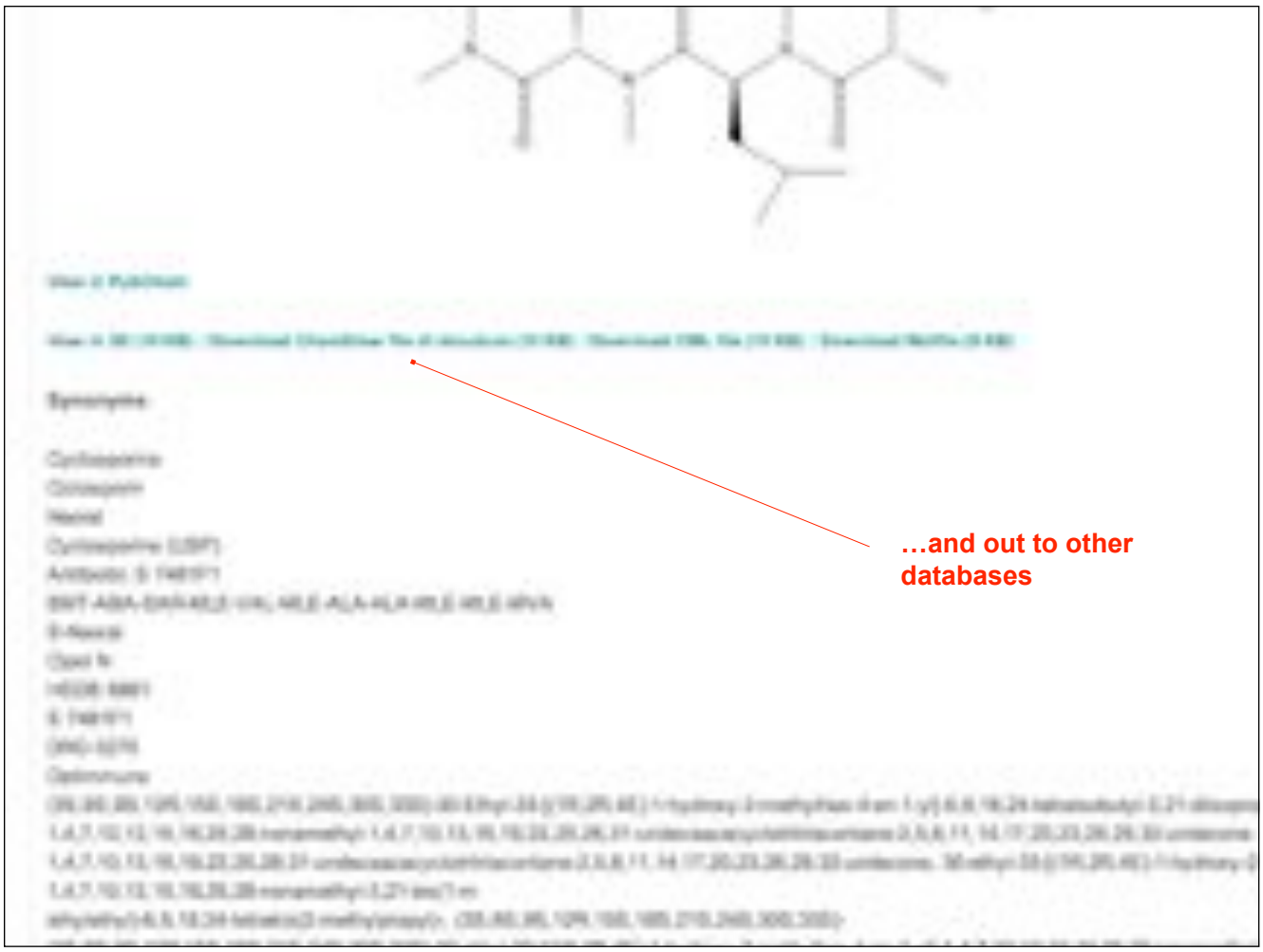
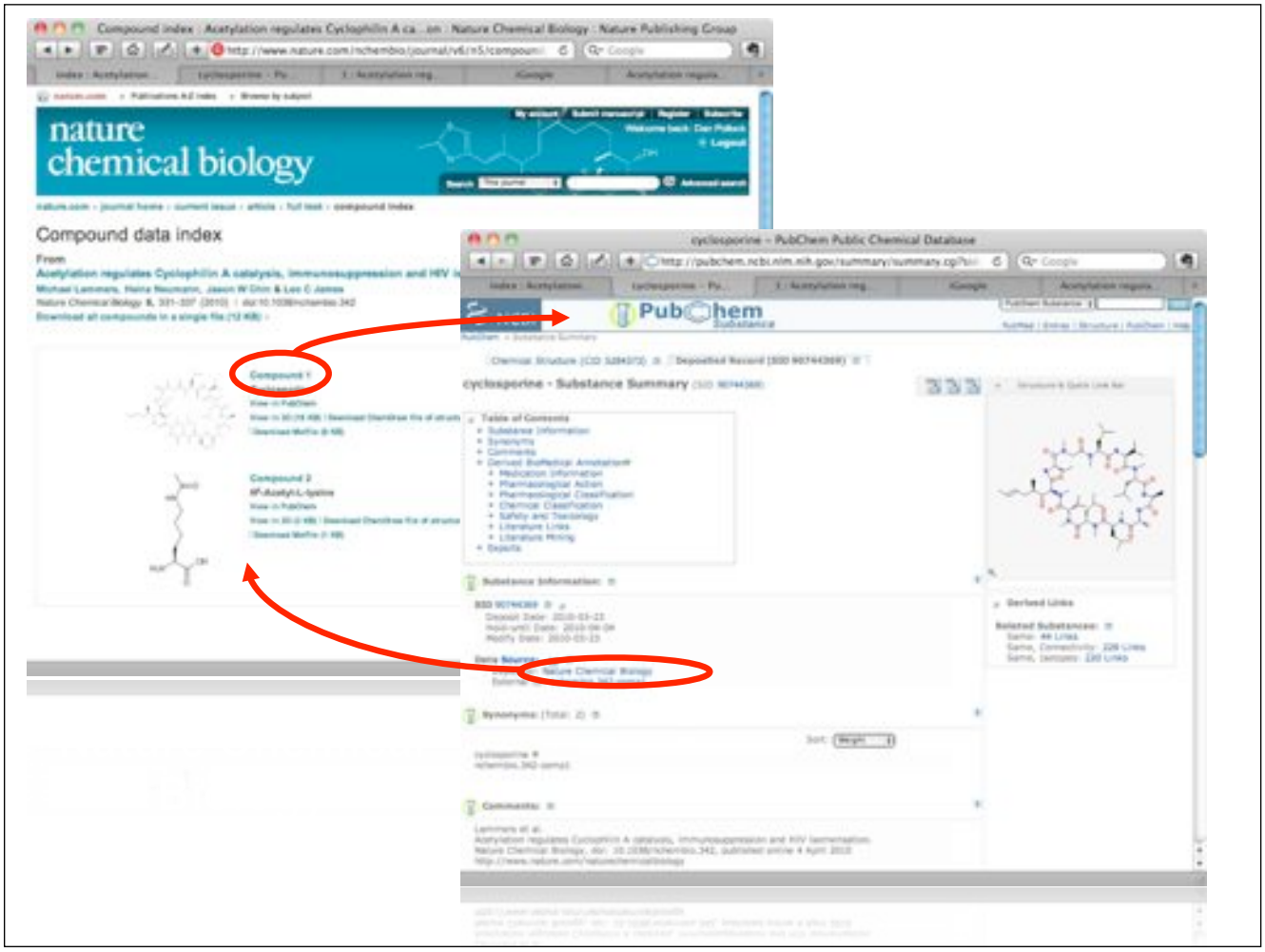
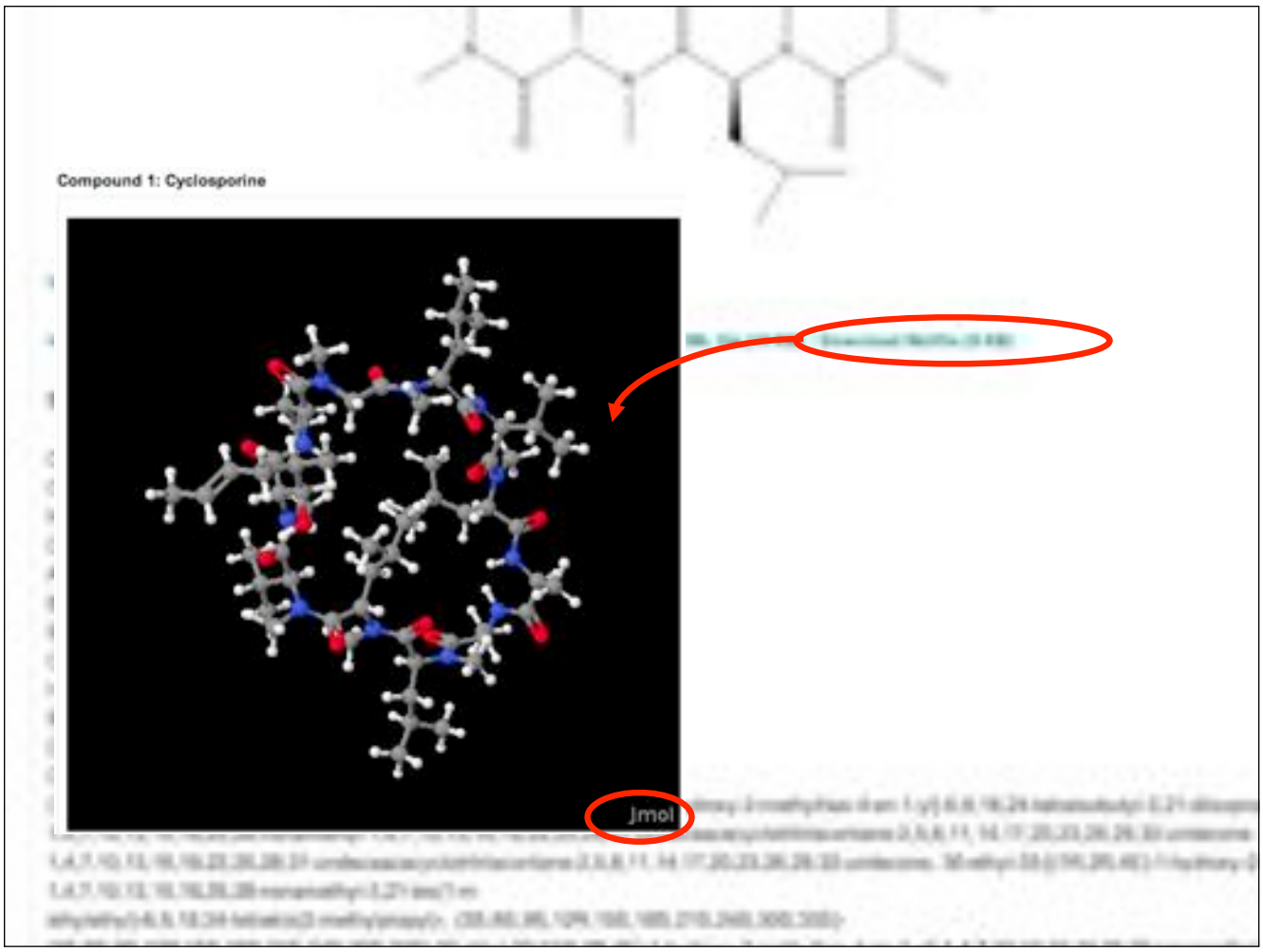

…and out to other databases


nature chemical biology
Compound data index
cyclosporine - PubChem Public Chemical Database
cyclosporine - Substance Summary

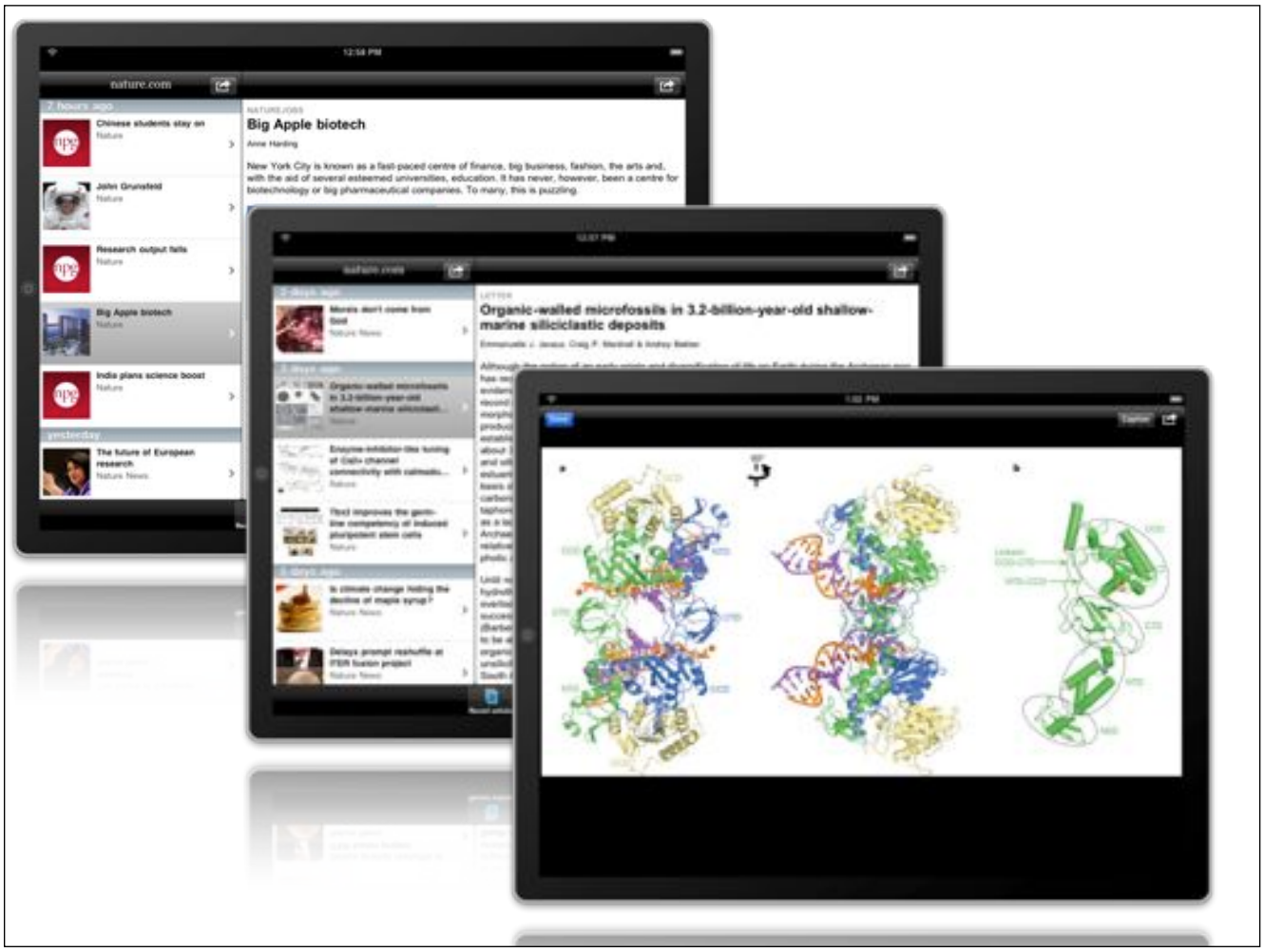


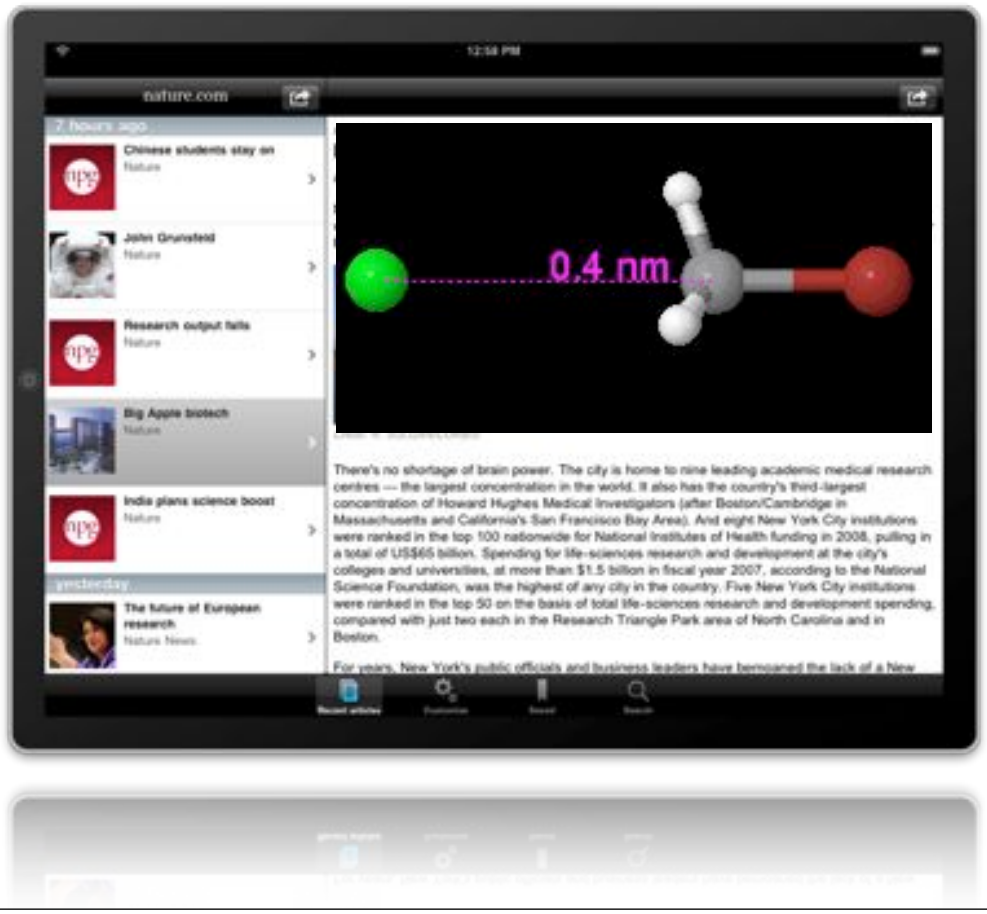
Article Improvement Project

- The article as a hub
- Modular framework
- From electronic print to digitally-optimised
- On-going
- Roll-out through 2010 and early 2011

Just a beginning


nature.com
An epistatic ratchet constrains the direction of glucocorticoid receptor evolution
Jamie T. Bridgham et al.


nature.com
Big Apple biotech
Anne Harding
Organic-walled microfossils in 3.2-billion-year-old shallow-marine siliciclastic deposits



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