Table 1 The divergence of the αA-crystallin genes of the human, hamster and blind mole rat relative to that of the mouse gene

	Exon 1	Intron 1	Insert exon
Human	94	47	86
Hamster	95	59	99
Mole rat	90	49	93

Divergence is shown by comparing nucleotide sequences for the coding region of the first exon, the IE and the intervening sequence between them. Values given for coding regions represent the percentage of positions at which the nucleotides are identical; values for intron sequences represent the percentage of matched residues after alignment by the NUCALN program[22] using default values for all parameters.

accumulating changes at the same rate as pseudogenes (4.5×10^{-9} substitutions per site per year)[17], we estimate that the human gene stopped using the IE some 30 to 40 million years ago. Interestingly, the human genome contains pseudogenes for two other lens proteins, both γ-crystallins with functional counterparts in rats[18]. This silencing of genes and an exon may have been triggered by similar environmental changes resulting in altered requirements for lens proteins.

The IE was acquired by an extremely well conserved, tissue-specific gene, either by exon shuffling or by the development of functional splicing signals within an intron. Its transformation into a pseudo-exon shows that part of a functional gene can be released from evolutionary pressures while the rest remains stringently conserved. This process may have occurred many times in the evolutionary history of modern proteins, but is only rarely glimpsed now.

We thank Dr Graeme Wistow, Luke Pallansch and Thomas Lietman for discussion and Dawn Chicchirichi for typing the manuscript.

Received 19 October 1988; accepted 6 January 1989.

1. Gilbert, W. *Nature* 271, 501–502 (1978).
2. Blake, C. C. F. *Int. Rev. Cytol.* 93, 149–185 (1985).
3. Holland, S. K. & Blake, C. C. F. *Biosystems* 20, 181–206 (1987).
4. Breitbart, R. E., Andreadis, A. & Nadal-Ginard, B. *A. Rev. Biochem.* 56, 467–495 (1987).
5. de Jong, W. W., van Amelsvoort, A. M., van der Ouderaa, F. J. & Bloemendal, H. *Nature new Biol.* 246, 233–236 (1973).
6. de Jong, W. W., Zweers, A., Versteeg, M. & Nuy-Terwindt, E. C. *Eur. J. Biochem.* 141, 131–140 (1984).
7. Cohen, L. H., Westerhuis, L. W., de Jong, W. W. & Bloemendal, H. *Eur. J. Biochem.* 89, 259–266 (1978).
8. Cohen, L. H., Westerhuis, L. W., Smits, D. P. & Bloemendal, H. *Eur. J. Biochem.* 89, 251–258 (1978).
9. de Jong, W. W., Cohen, L. H., Leunissen, A. M. & Zweers, A. *Biochem. biophys. Res. Commun.* 96, 648–655 (1980).
10. Hendriks, W., Sanders, J., de Leij, L., Ramaekers, F., Bloemendal, H. & de Jong, W. W. *Eur. J. Biochem.* 174, 133–137 (1988).
11. King, C. R. & Piatigorsky, J. *Cell* 32, 707–712 (1983).
12. van den Heuvel, R., Hendriks, W., Quax, W. & Bloemendal, H. *J. molec. Biol.* 185, 273–284 (1985).
13. McDevitt, D. S., Hawkins, J. W., Jaworski, C. J. & Piatigorsky *J. expl. Eye Res.* 43, 285–291 (1986).
14. Thompson, M. A., Hawkins, J. W. & Piatigorsky, J. *Gene* 56, 173–184 (1987).
15. Hendriks, W., Leunissen, J., Nevo, E., Bloemendal, H. & de Jong, W. W. *Proc. natn. Acad. Sci. U.S.A.* 84, 5320–5324 (1987).
16. Perler, F. *et al. Cell* 20, 555–566 (1980).
17. Li, W.-H., Gojobori, T. & Nei, M. *Nature* 292, 237–239 (1981).
18. Meakin, S. O., Du, R. P., Tsui, L. C. & Breitman, M. L. *Molec. cell. Biol.* 7, 2671–2679 (1987).
19. de Jong, W. W. & Goodman, M. *Sonderdruck Z. Saugetierkunde* 47, 257–276 (1982).
20. de Jong, W. W., Terwindt, E. C. & Bloemendal, H. *FEBS Lett.* 58, 310–313 (1975).
21. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* 74, 5463–5467 (1977).
22. Wilbur, W. J. & Lipman, D. J. *Proc. natn. Acad. Sci. U.S.A.* 80, 726–730 (1983).

Dynamical transition of myoglobin revealed by inelastic neutron scattering

Wolfgang Doster*, Stephen Cusack† & Winfried Petry‡

* Physik Department E13, Technischen Universität München,
D-8046 Garching, FRG
† EMBL Grenoble Outstation, Institut Laue-Langevin, 156X,
38042 Grenoble Cedex, France
‡ Institut Laue-Langevin, 156X, 38042 Grenoble Cedex, France

Structural fluctuations in proteins on the picosecond timescale have been studied in considerable detail by theoretical methods such as molecular dynamics simulation[1,2], but there exist very few experimental data with which to test the conclusions. We have used the technique of inelastic neutron scattering to investigate atomic motion in hydrated myoglobin over the temperature range 4–350 K and on the molecular dynamics timescale 0.1–100 ps. At temperatures below 180 K myglobin behaves as a harmonic solid, with essentially only vibrational motion. Above 180 K there is a striking dynamic transition arising from the excitation of non-vibrational motion, which we interpret as corresponding to torsional jumps between states of different energy, with a mean energy asymmetry of 12 kJ mol^{-1}. This extra mobility is reflected in a strong temperature dependence of the mean-square atomic displacements, a phenomenon previously observed specifically for the heme iron by Mössbauer spectroscopy[3–5], but on a much slower timescale (10^{-7} s). It also correlates with a glass-like transition in the hydration shell of myoglobin[6] and with the temperature-dependence of ligand-binding rates at the heme iron, as monitored by flash photolysis[7]. In contrast, the crystal structure of myoglobin determined down to 80 K shows no significant structural transition[8–10]. The dynamical behaviour we find for myoglobin (and other globular proteins) suggests a coupling of fast local motions to slower collective motions, which is a characteristic feature of other dense glass-forming systems.

Inelastic neutron scattering is a spectroscopic technique which can be used to study protein motions on exactly the same timescale (0.1–100 ps) as is now widely accessible by computer simulation[11]. Because of the anomalously large incoherent neutron cross-section of the ^{1}H nucleus, the method specifically probes the motion of hydrogen atoms. As hydrogens are abundant and are uniformly distributed in proteins, the method gives a global view of protein dynamics. The quantity measured experimentally is the incoherent dynamic structure factor $S(q, \omega)$, where $\hbar q$ and $\hbar\omega$ are respectively the momentum and energy transfers between system and incident neutron. $S(q, \omega)$ is the Fourier transform of the time-correlation function of the density fluctuations in a system and can be directly calculated from the results of a molecular dynamics simulation. For samples of myoglobin hydrated with D_2O, we have measured as a function of temperature both the elastic intensity $S(q, \omega=0)$, which gives information on the geometry of motions, and $S(q, \omega>0)$, which gives the timescale (or spectrum) of the corresponding diffusive and vibrational motion[12,13].

Between 4 K and 180 K the elastic intensity of myoglobin has the gaussian form expected for a harmonic solid whose vibrational motion can be described by a Debye–Waller factor, that is, $S(q, \omega=0)=\exp(-q^2\langle\Delta x^2\rangle)$, where the mean-square displacement $\langle\Delta x^2\rangle$ is proportional to temperature (except for quantum effects at very low temperatures). But near 200 K an extra decrease in the elastic intensity at low q is observed, indicating the excitation of new degrees of freedom. The geometry of these motions gives rise to a transition to a non-gaussian elastic intensity (Fig. 1). As the deviation from gaussian behaviour increases with temperature, we can conclude that the motion involves jumping of hydrogens to distinct sites of different energy. The simplest model accounting for these observations involves jumps between two sites separated by a distance d and free energy ΔG (Fig. 2). The powder-averaged normalized elastic

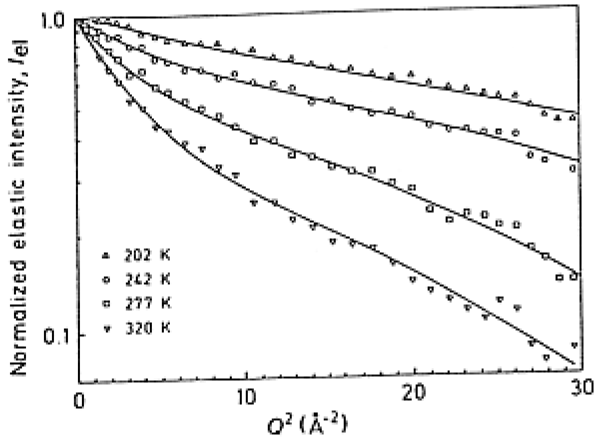

Fig. 1 The elastic intensity $I_{el}(q) = S(q, \omega \approx 0)$ was determined with a fixed window method at a resolution of 10 µeV (FWHM) using the backscattering spectrometer[26] IN13 at the Institut Laue-Langevin in Grenoble, France with an incident wavelengh of 2.23 Å. The sample was continuously heated from 4 K to 320 K over a period of 24 h. Raw data were corrected for cell scattering and detector response and normalized to unity at $q = 0$. The lines are fits to equation (1). The sample was ~400 mg sperm-whale myoglobin powder (Sigma) hydrated to 0.38 g D_2O per g protein, at which level the protein is essentially fully hydrated. The D_2O contributes ~5% to the total scattering cross-section; the neutron spectrum is thus dominated by scattering from unexchanged protons in the protein (CH, CH_2 and CH_3 and a few inaccessible NH) which reflect internal and surface protein motions.

intensity is then given by[14]

$$S(q, 0) = e^{-q^2\langle\Delta x^2\rangle_G}\left\{1 - 2p_1p_2\left(1 - \frac{\sin(qd)}{qd}\right)\right\} \qquad (1)$$

The first term describes the gaussian contribution to the mean-square displacement (below 180 K $\langle\Delta x^2\rangle_G = \langle\Delta x^2\rangle_{\mathrm{vib}}$) and the term in brackets denotes the elastic incoherent structure factor of the two-state model. The variables p_1 and p_2 denote the probability of finding a hydrogen in the ground or excited state respectively, with $p_2/p_1 \propto \exp(-\Delta G/RT)$. The second factor contributes to the q-dependence of $S(q, 0)$ essentially only for $qd < 1$. The hydrogen mean-square displacement is given by

$$\langle\Delta x^2\rangle = -\left(\frac{d\ln\{S(q, 0)\}}{d(q^2)}\right)_{q=0}$$
$$= \langle\Delta x^2\rangle_G + \frac{p_1p_2d^2}{3} \qquad (2)$$

which is the initial slope of the curves in Fig. 1. Least-squares fits of equation (1) to the data (Fig. 1) give p_1p_2, $\langle\Delta x^2\rangle$ and $\langle\Delta x^2\rangle_G$ as a function of temperature (Fig. 2). A van't Hoff plot of p_2/p_1 gives an energy asymmetry of $\Delta H = 12(\pm 2)$ kJ mol^{-1} and entropy $\Delta S/R = 3.0(\pm 0.1)$. The value of the jump distance d is found to be 1.5(±0.1) Å. The magnitude of this value would indicate the involvement of torsional degrees of freedom and large amplitude, fast dihedral angle fluctuations are indeed observed in molecular dynamics simulations of proteins[15]. As discussed more fully elsewhere, the parameters given above are dependent on hydration. In particular, the excess entropy of the excited state is significantly reduced on dehydration and the distance d drops to 1.1 Å. Furthermore at low and intermediate levels of hydration, $\langle\Delta x^2\rangle_G$ is dominated by the vibrational contribution $\langle\Delta x^2\rangle_{\mathrm{vib}}$, whereas in the high-hydration data presented here there is an additional gaussian process, $\langle\Delta x^2\rangle_G = \langle\Delta x^2\rangle_{\mathrm{vib}} + \langle\Delta x^2\rangle_\alpha$. The resulting $\langle\Delta x^2\rangle_\alpha$ is also shown in Fig. 2.

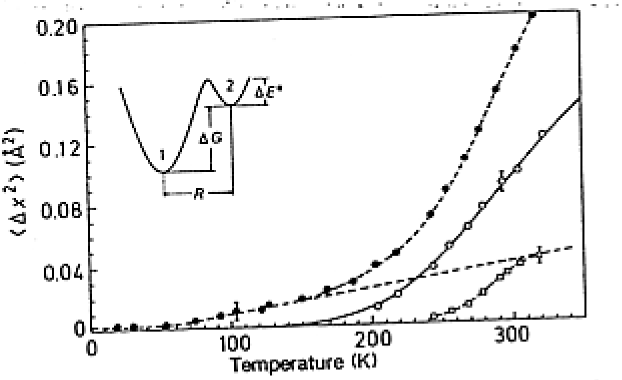

Fig. 2 Average mean-square hydrogen displacements obtained from the analysis of $I_{el}(q)$ assuming an asymmetric two-state model. The full circles give the total mean-square displacement $\langle\Delta x^2\rangle$ defined by equation (2). The solid line shows $\langle\Delta x^2\rangle_\beta = p_1p_2d^2/3$ obtained using the parameters in the text and the open circles give the corresponding experimental points. The squares show $\langle\Delta x^2\rangle_\alpha$ (see text). The dashed straight line is the vibrational contribution $\langle\Delta x^2\rangle_{\mathrm{vib}}$ extrapolated linearly from low temperature. A symmetrical model with two excited states may be more appropriate for torsional motion. Such a model does indeed fit the data equally well, but the derived parameters (apart from the entropy, which is lower) are not significantly different. Jump models with multiple ground states are excluded by the data. Quantum effects are important below 50 K, leading to a temperature-independent $\langle\Delta x^2\rangle$ of 0.005 (±0.005) Å^2.

The extra loss in elastic intensity is compensated by neutrons scattered into a broad quasielastic spectrum between 0 and 3 meV. To derive the actual shape of this spectrum requires a careful subtraction of the vibrational background (manuscript in preparation; see also ref. 16). In Fig. 3 we show the resultant quasielastic spectra combining data from two spectrometers with different energy resolutions and the inset shows the corresponding correlation functions. Two spectral components with different shape and temperature dependence can clearly be recognized, a fast β-process and a slower α-process. The increase with temperature of the intensity of the broad β-line is consistent with local jumps between two states of energy asymmetry of 12 kJ mol^{-1}. Surprisingly however, the linewidth is temperature-independent with a value of 1.5-2 meV (corresponding to a correlation time of ~0.3-0.5 ps, see inset to Fig. 3). Within the framework of the two-state model, this would imply that the activation energy ΔE^*, essentially the depth of the well in the excited state, is smaller than RT (<1.6 kJ mol^{-1}). Qualitatively similar behaviour of the quasielastic spectrum has been obtained with the polymeric glass polybutadiene[17].

The linewidth of the α-relaxation by contrast broadens with increasing temperature. Below 240 K this component is not well resolved and therefore contributes to the elastic intensity. The gaussian mean-square displacement $\langle\Delta x^2\rangle_\alpha$ (Fig. 2) is related to this process. The shape of the α-relaxation is clearly not lorentzian but is reasonably well fitted by a Cole-Davidson function[18] often used to parameterize the α-process in liquids and polymers:

$$S(q, \omega) = -A_1(q)\,\mathrm{Im}\{(1 + i\omega\tau_0)^{-b}\}/\omega \qquad (3)$$

This formula implies a power-law dependence at high frequencies with exponent $-(1+b)$, (a lorentzian has $b = 1$). We find a value for b of 0.25(±0.1), which is temperature independent between 250 K and 350 K (Fig. 3). The temperature-independent shape essentially excludes an explanation of this component in terms of a distribution of correlation times unless we assume that the corresponding distribution of activation energies varies with temperature in a unique way. The shape of the total spectrum (α and β) however is qualitatively consistent with the results derived from a mode-coupling theory of the liquid-glass transition in a hard core liquid[19-22]. The α-process in this theory is a collective effect, its shape arising as a result of non-linear coupling between density fluctuations. The β-process corresponds to local motions of atoms in the cage formed by their neighbours. Our data therefore demonstrate that diffusive motions in a protein resemble those in other close-packed sys-

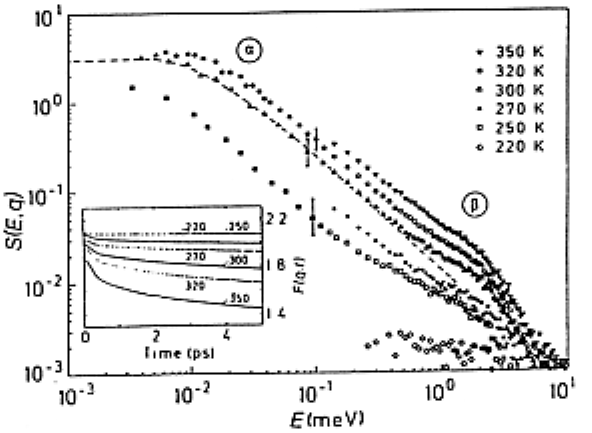

Fig. 3 Log/log plot of quasielastic neutron spectra obtained by combining the results of IN13 (filled symbols) and the time-of-flight spectrometer IN6 (open symbols)[26]. IN6 has a gaussian resolution function ($\sigma = 50\ \mu$eV) and an incident wavelength of 5.1 Å. In addition to the usual corrections, a vibrational background has been subtracted at each temperature; this was obtained by scaling the inelastic scattering measured at 180 K or 100 K, assuming harmonic behaviour. The IN6 data represent a single scattering angle (58.5°); the IN13 data have been averaged over the q-range of IN6 (2 Å^{-1}) to improve the statistics. Shape and linewidth of the spectra are roughly independent of q. The data from the spectrometers were matched at the overlap energy of 0.1 meV. Spectra are not corrected for multiple scattering. The effect of instrumental resolution is small within the energy range presented here. The dashed line represents equation (3) convoluted with the resolution function of IN13, with $b = 0.25$ and $\langle\tau\rangle = b\tau_0 = 2.10^{-11}$ s. The β-process has a shape intermediate between gaussian and lorentzian and an estimated correlation time of 0.35 ps. Inset: correlation functions obtained from the IN6 quasielastic spectra by Fourier transform and deconvolution of the instrumental resolution function. The overall decrease in intensity with increasing temperature is due to the decrease in the elastic peak which was not subtracted out and contributes a flat background to the correlation functons.

tems dominated by hard-core interactions. But some unique features have to be explained in terms of protein structure: the exponent 0.25 is unusually small (close to $1/f$ noise) and the non-gaussian scattering law, indicating jumps between distinct sites of different energy, suggests that the geometry of the local motion is highly constrained by strong interactions along the chain. A similar asymmetric two-state model has been proposed to explain the microwave absorption of the main-chain in lysozyme and haemoglobin[23]. The corresponding energy asymmetry is almost identical to our result. In this connection we remark that neutron data on hydrated lysozyme powders (not shown) behave very similarly to those from myoglobin, suggesting that the dynamic phenomena described here are a general property of globular proteins. The temperature dependence of the hydrogen mean-square displacement (Fig. 2) resembles that derived for the heme iron by Mössbauer spectroscopy[3-5]. This correlation indicates that the picosecond fluctuations observed with neutron scattering could couple to the heme group and are likely to be an important component in the decrease of the Lamb-Mössbauer factor above 200 K (ref. 5), as well as in the slower motions resolved in the Mössbauer spectrum (down to 10^{-7} s). Further discussion of this point awaits the current re-evaluation of new and more accurate Mössbauer data[24].

Finally we suggest that an important test of the molecular dynamics simulation technique would be to reproduce the temperature dependence of the picosecond protein dynamics reported here. This would also provide insight into the specific atomic motions involved. A step in this direction is the recent theoretical analysis of multiple conformational states in myoglobin[25].

This project is supported by a EMBO short-term fellowship and a grant from the Deutsche Forschungsgemeinschaft. We thank Dr A. Dianoux for assistance on IN6 and discussions on the manuscript. Assistance and useful suggestions by Dr Fujara, Professor Dr E. Lüscher and F. Post are also gratefully acknowledged.

Received 28 September 1988; accepted 9 January 1989.

1. McCammon, J. A. *Rep. Prog. Phys.* 47, 1–46 (1984).
2. Levy, R. M. *et al. Biophys. J.* 48, 509–518 (1985).
3. Parak, F. *et al. FEBS Lett.* 117, 368–372 (1980).
4. Keller, H. & Debrunner, P. *Phys. Rev. Lett.* 45, 68–71 (1980).
5. Bauminger, E. R., Cohen, S. G., Nowik, I., Ofer, S. & Yariv, J. *Proc. natn. Acad. Sci. U.S.A.* 80, 736–740 (1983).
6. Doster, W., Bachleitner, A., Dunau, R., Hieble, M. & Lüscher, E. *Biophys. J.* 50, 213–219 (1986).
7. Austin, R. H., Beeson, K. W., Eisenstein, L., Frauenfelder, H. & Gunsalcs, I. C. *Biochemistry* 14, 5355–5373 (1975).
8. Frauenfelder, H., Petsko, G. A. & Tsernoglou, D. *Nature* 280, 558–563 (1979).
9. Hartmann, H. *et al. Proc. natn. Acad. Sci. U.S.A.* 79, 4967–4971 (1982).
10. Parak, F. *et al. Eur. Biophys. J.* 15, 237–249 (1987).
11. Cusack, S. in *The Enzyme Catalysis Process: Energetics, Mechanism and Dynamics* (eds Cooper, A. & Houben, J. (NATO ASI series, Plenum, London, in the press).
12. Springer, T. in *Quasielastic Neutron Scattering for the Investigation of Diffusive Motions in Solids and Liquids* (Springer Tracts in Modern Physics, Vol. 64, 1972).
13. Bee, M. in *Quasielastic Neutron Scattering: Principles and Applications in Solid-state Chemistry, Biology and Materials Science* (Hilger, Bristol, 1988).
14. Stöckli, H., Furrer, A., Schoenenberger, Ch., Meier, B. H., Ernst, R. R. & Anderson, I. *Physica* 136B, 161–164 (1986).
15. Levitt, M. *J. molec. Biol.* 168, 621–657 (1983).
16. Cusack, S., Smith J., Finney, J., Tidor, B. & Karplus, M. *J. molec. Biol.* 202, 903–908 (1988).
17. Frick, B., Richter, D., Petry, W. & Buchenau, U. *Z. Phys.* B70, 1–3 (1988).
18. Davidson, D. W. & Cole, R. H. *J. chem. Phys.* 18, 1417–1419 (1950).
19. Bengtzelius, W., Götze, W. & Sjölander, A. *J. Phys.* C17, 5915–5933 (1984).
20. Götze, W. & Sjögren, L. *J. Phys.* C20, 879–894 (1987).
21. Götze, W. & Sjögren, L. *J. Phys.* C21, 3407–3471 (1988).
22. Doster, W., Cusack, S. & Petry, W. Proc. ILL Workshop *Dynamics of Disordered Materials*, Grenoble, 1988.
23. Poglitsch, H., Kremer, F. & Genzel, L. *J. molec. Biol.* 173, 137–142 (1984).
24. Parak, F., Heidemeier, J. & Nienhaus, G. U. *Hyperfine Interactions* 40, 147–158 (1988).
25. Elber, R. & Karplus, M. *Science* 235, 318–321 (1987).
26. *Neutron Research Facilities at the ILL High-Flux Reactor* (Institut Laue-Langevin, Grenoble, 1988).

GUIDE TO AUTHORS

Please follow these guidelines so that your manuscript may be handled expeditiously.

Nature is an international journal covering all the sciences. Contributors should therefore bear in mind those readers for whom English is a second language and those who work in other fields. Please write clearly and simply, avoiding unnecessary technical terminology. *Nature's* staff will edit manuscripts to those ends if necessary. Contributors should check their proofs carefully.

Because of the competition for space, many of the papers submitted for publication cannot be accepted. For this reason, and because brevity is a great assistance to readers, papers should be as brief as is consistent with intelligibility. Please note that one printed page of *Nature*, without diagrams or other interruptions of the text, has fewer than 1,300 words.

CATEGORIES OF PAPER

Review Articles survey recent developments in a field. Most are commissioned, but suggestions are welcome in the form of a one-page synopsis addressed to the Reviews Coordinator. Length is negotiable in advance but must not exceed six pages of *Nature*.

Articles are research reports whose conclusions are of general interest and which are sufficiently rounded to be a substantial advance in understanding. They should not have more than 3,000 words of text or more than six display items (figures and tables) and should not occupy more than five pages of *Nature*.

Articles should be accompanied by a heading of 50 – 80 words written to advertise their contents in general terms, to which editors will pay particular attention. A heading (printed in italic type) is not an abstract and should not usually contain numbers or measurements. The study should be introduced in more detail in the first two or three paragraphs, which should also briefly summarize its results and implications.

Articles may contain a few subheadings of two or three words. The meaning of the text should not depend on the subheadings, whose function is to break up the text and to point to what follows. There should be fewer than 50 references.

Letters to Nature are short reports of outstanding novel findings whose implications are general and important enough to be of interest to those outside the field. Letters should not have more than 1,000 words of text or more than four display items and should not occupy more than two pages of *Nature*. The first paragraph should describe, in not more than 150 words, the origins and chief conclusions of the study. Letters should not have subheadings or more than 30 references.

Commentary articles deal with issues in, or arising from, research that are also of interest to readers outside research. Some are commissioned, most are unsolicited. They are normally between one and four pages of *Nature* in length.

News and Views articles are intended to inform non-specialist readers about a recently published advance. Suggestions should be made to the News and Views Editor. Illustrations are welcome. Proposals for meeting reports should be agreed in advance.

Scientific Correspondence is for the discussion of scientific matters, including contributions published in *Nature*. Priority is given to contributions of less than 500 words and five references. Figures are welcome.

GENERAL

All contributions submitted for publication in *Nature* should conform with these rules.

Manuscripts should be typed, double-spaced, with a good-quality printer, on one side of the paper only. Four copies are required, each accompanied by lettered artwork. Four copies of half-tones should be included. Reference lists, figure legends and so on should be on separate sheets, all of which should be double-spaced and numbered. Copies of relevant manuscripts in press or submitted for publication elsewhere should be included, clearly marked as such. Revised and resubmitted manuscripts should also be clearly marked as such and labelled with their reference numbers.

Titles should say what the paper is about with the minimum of technical terminology and in fewer than 80 characters. Authors should avoid active verbs, numerical values, abbreviations and punctuation and should include one or two key words for indexing purposes.

Artwork should be marked individually and clearly with the author's name, figure number and, when known, manuscript reference number. Original artwork should be unlettered. Ideally, no figure should be larger than 28 by 22 cm. Figures with several parts are permitted only if the parts are closely related, either experimentally or logically. Suggestions for cover illustrations, with captions, are welcome. Original artwork (and one copy of the manuscript) will be returned when a manuscript cannot be published.

Colour Artwork. A charge of £500 a page is made for 4-colour figures. Inability to meet these costs will not prevent the publication of essential colour figures if the circumstances are explained.

Figure legends must not exceed 300 words and ideally should be much shorter, and should use telegraphic form wherever possible. The figure should be described first, then, briefly, the method. Reference to a method described elsewhere is preferable to a full description. Methods should not be described in detail in the text.

References should be numbered sequentially as they appear in the text, followed by those in tables and finally those in the figure legends. Reference numbers apply only to papers published or in the press, and a different number should be given to each paper cited. All other forms of reference (including unrefereed abstracts) should be included in the text as personal communication, manuscript in preparation or preprint (with number and institution where appropriate). Text should not be included in the reference list. References should be abbreviated according to the *World List of Scientific Periodicals* (fourth edition, Butterworth, London, 1963 – 65). The first and last page numbers should be cited. Reference to books should clearly indicate the publisher and the date and place of publication.

Abbreviations, symbols, units and Greek letters should be identified the first time they are used. Acronyms should be avoided as much as possible and, when used, defined. Editors will shorten words if necessary. In case of doubt, SI units should be used.

Footnotes should not be used except for changed addresses or to identify the corresponding author if different from the first-named.

Acknowledgements should be brief. Grant numbers and contribution numbers are not allowed.

***Submission.** All manuscripts may be submitted to either London or Washington. They should not be addressed to editors by name. Manuscripts or proofs sent by air courier to London should be declared as 'manuscripts' and 'value $5' to [illegible] imposition of import duty and value-added tax (VAT).*