Title

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When two γ rays that are emitted successively in nuclear de-excitation are detected in coincidence, the coincidence counting rate, $W(\theta)$, may depend strongly on the angle θ between their directions of propagation. For the 173-247 keV γ-ray cascade in ^{111}Cd following the decay of ^{111}In, shown in Fig. 1, $W(\theta)$ is given by[1]:

$$W(\theta) = \frac{1}{\tau} e^{-t/\tau} [1 + A_2 P_2 (\cos \theta)] \quad , \qquad (1)$$

where P_2 is the Legendre polynomial $(3\cos^2\theta - 1)/2$, τ is the mean life-time of the intermediate nuclear state[2], t is the time interval between emission of the two γ rays, and the coefficient $A_2 = -0.20$.

The angular correlation of the 173-247 keV γ ray cascade in ^{111}Cd can be perturbed by the interaction of the nuclear moments in the intermediate state with fluctuating external fields. In this case, the coefficient of P_2 can be conveniently written as $A_2 G_2(t)$ where $G_2(t)$ is an attenuation coefficient. A study of the perturbed angular correlation of γ radiation can then provide a measure of the nuclear relaxation time and thereby yield the rotational correlation time, τ_c, of a molecule to which the radioactive nucleus is bound. Although angular correlations have been employed for some time to study nuclear properties[3], and have more recently been applied to studies of the properties of solids[4], angular correlations have not previously been applied to the study of macromolecules in solution.

In this note we report the observation of effects of molecular conformation on the angular correlation pattern of γ rays following the decay of ^{111}In when this isotope is bound to binding sites of biological macromolecules in solution. The use of a radioactive nucleus as a "rotational tracer" to label biomolecules offers the possibility of obtaining the information available from other labelling techniques[5-7] with the sensitivity, instrumental simplicity and *in-vivo* applicability of radioactive tracer techniques.

Cadmium metal (12.75% ^{111}Cd) was irradiated with 10-MeV protons in the Berkeley 88-inch cyclotron, producing 2.8-day[8] ^{111}In by the (p,n) reaction. After chemical separation from the Cd target, the ^{111}In was added as In^{+3} to aqueous solutions containing bovine serum albumin (BSA) or polyglutamic acid (PGA). In these experiments commercially-available BSA (Mann Research Labs, twice crystallized) and PGA (DP530, Pilot Chemical Co.) were used without further purification. The angular correlation measurements were made with a 4-detector delayed-coincidence spectrometer[9] using 0.2 cc liquid samples.

For the unperturbed angular correlation of the ^{111}Cd cascade following ^{111}In decay, the coefficient $A_2G_2(t)$ should be independent of the delay time t and equal to -0.20. Figure 2 is a plot of the anisotropy, $A(t) = 1 - W(180)/W(90) \cong 0.30\ G_2(t)$ vs t for this cascade following the decay of $^{111}In^{3+}$ ion in the presence of BSA at pH 5.7. The angular correlation is strongly perturbed. When the pH of this solution is reduced to 2.8, A(t) approaches more closely the unperturbed behavior that is observed following the decay of In^{3+} ion in solution[1]. Since BSA undergoes a conformational transition between pH 5.7 and 2.8[10], the changes in the angular

correlation may reflect changes in the effective rotational correlation time at the binding site(s) of ^{111}In, or they may simply reflect a change in the effective binding constant. At pH 5.7 the protein may be more rigid, giving a larger value for τ_c, and a larger perturbation in the angular correlation. The particular time dependence found for $G_2(t)$ in this case is very similar to that observed in solids[11]. We note that only *time-independent* (quadrupole) interactions are involved for this (pH 5.7) sample. Although τ_c is also sensitive to the macroscopic viscosity, the viscosities of these solutions at pH 5.7 and 2.8 measured with an Ostwald viscometer at 21.5°C are respectively 1.04±0.02 and 1.10±0.02 C.P. These values are nearly equal, and both are too small to affect G_2 significantly[11].

A comparison of the plots of $A(t)$ obtained with the BSA solution at pH 5.7, and a similar solution with 8M urea added is shown in Fig. 3. The effect of adding 8M urea is large, and in the direction expected for denaturation of the molecule leading to shorter effective values for τ_c, and thus smaller perturbations of the angular correlation times.

In this case the viscosity of the urea solution is 1.38±0.06 C.P. The effect of bulk viscosity alone should be small, quite different, and opposite to the direction of the observed changes.

Since the γ ray cascade observed in the daughter ^{111}Cd nuclei follows electron capture decay of ^{111}In on the binding sites, the possibility that aftereffects following decay might lead to detachment of Cd atoms from these sites is a major concern. To avoid detachment, the Cd^{2+} ions have to achieve a stable chemical configuration during the lifetime[8] of the 419-keV

state of ^{111}Cd ($T_{1/2} = 1.2\times10^{-10}$ sec). The displacement of $^{111}In^{3+}$ by Hg^{2+} and the competitive complexing of $^{111}In^{3+}$ by EDTA, dialysis of the BSA-In^{3+} solutions, and analogous qualitative experiments with PGA suggest that the radioactive isotope indeed binds to the macromolecules and reflects the effective molecular rotational correlation time at the binding site.

The radioactive rotational label technique shares a number of general features with other labeling techniques. Information on localized behavior of the macromolecule near the labeling site is usually available. The labels can be incorporated into interesting regions of macromolecules using selective chemical methods. Labels can be bound chemically to substrate or inhibitor molecules which subsequently interact with high specificity at active regions of enzymes or antibodies[12,13]. On the other hand, there is always uncertainty as to how much the label affects the system being observed. The compelling factor in developing this application is the extremely high sensitivity of angular correlations. In these experiments only 10^{10} nuclei of ^{111}In were used in each sample. The angular correlation method should find considerable further application to studies of biological macromolecules.

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FIGURE CAPTIONS

Fig. 1. The 173-247 keV γ-ray cascade in ^{111}Cd following the electron capture decay of ^{111}In (data from Ref. 8).

Fig. 2. Plot of the anisotropy A(t) as a function of delay time for a 1.4×10^{-4} M aqueous BSA solution at pH 5.7 (○) and pH 2.8 (●). The error bars indicate the statistical counting error. The uncertainty in the time scale is about 1%.

Fig. 3. Plot of the anisotropy A(t) as a function of delay time, t, for a 1.4×10^{-4} M aqueous BSA solution at pH 5.7 with no urea (○) and in the presence of 8M urea (●). The dashed line shows the behavior of A(t) as a function of t for an aqueous solution of $InCl_3$.

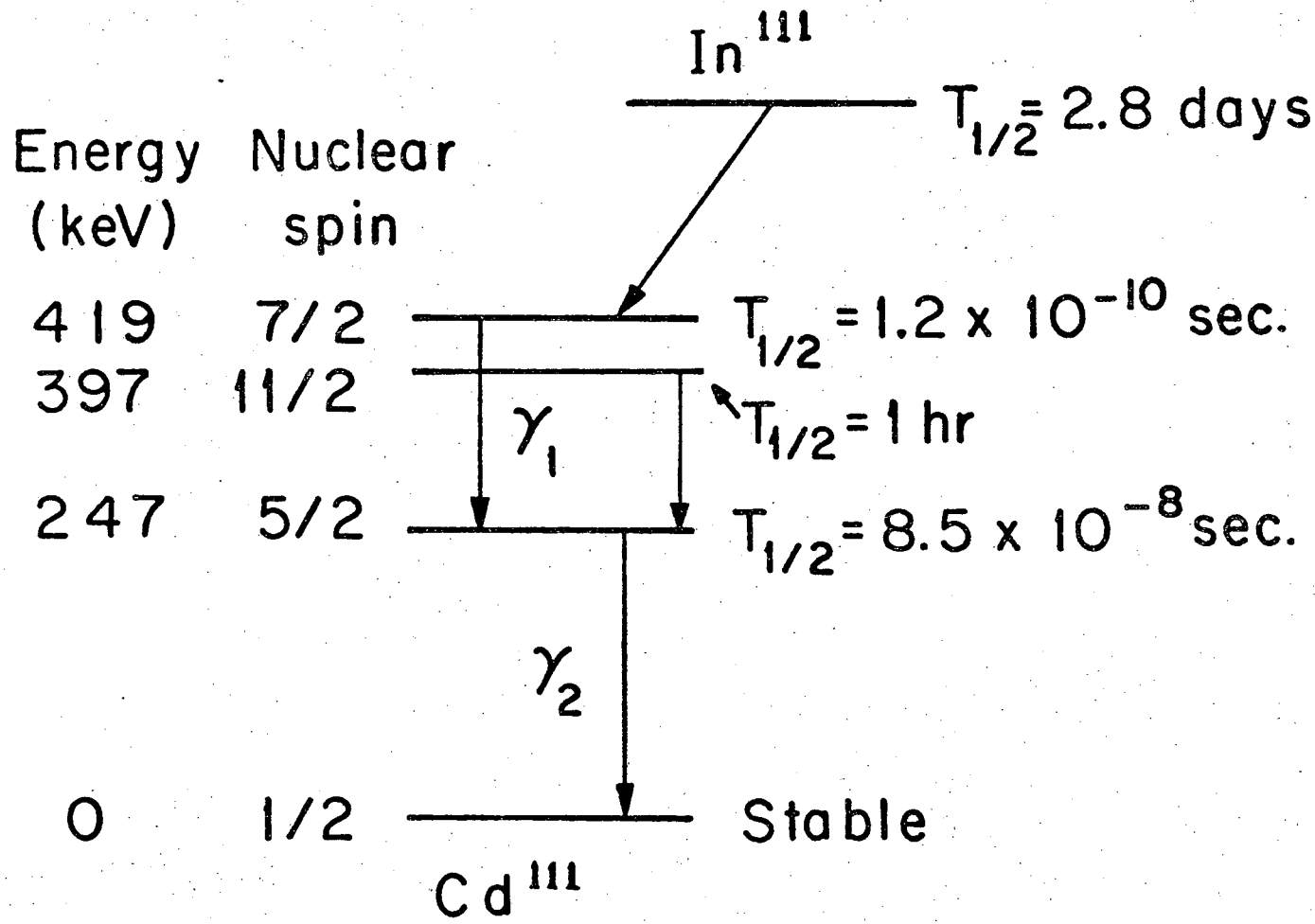

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Fig. 1

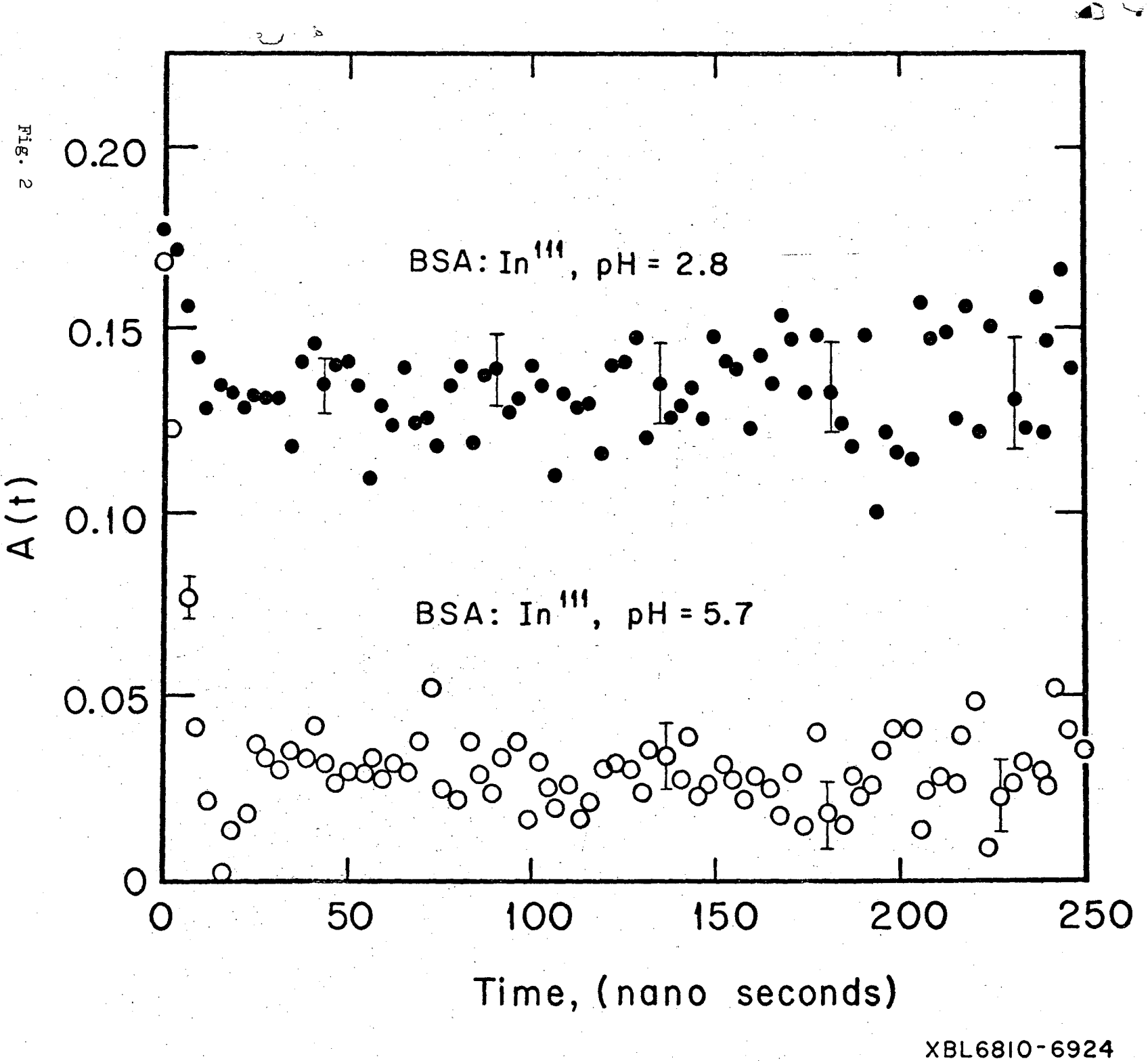
0.20
0.15
0.10
0.05
0
A(t)
BSA: In^{111}, pH = 2.8
BSA: In^{111}, pH = 5.7
0
50
100
150
200
250
Time, (nano seconds)
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Fig. 2

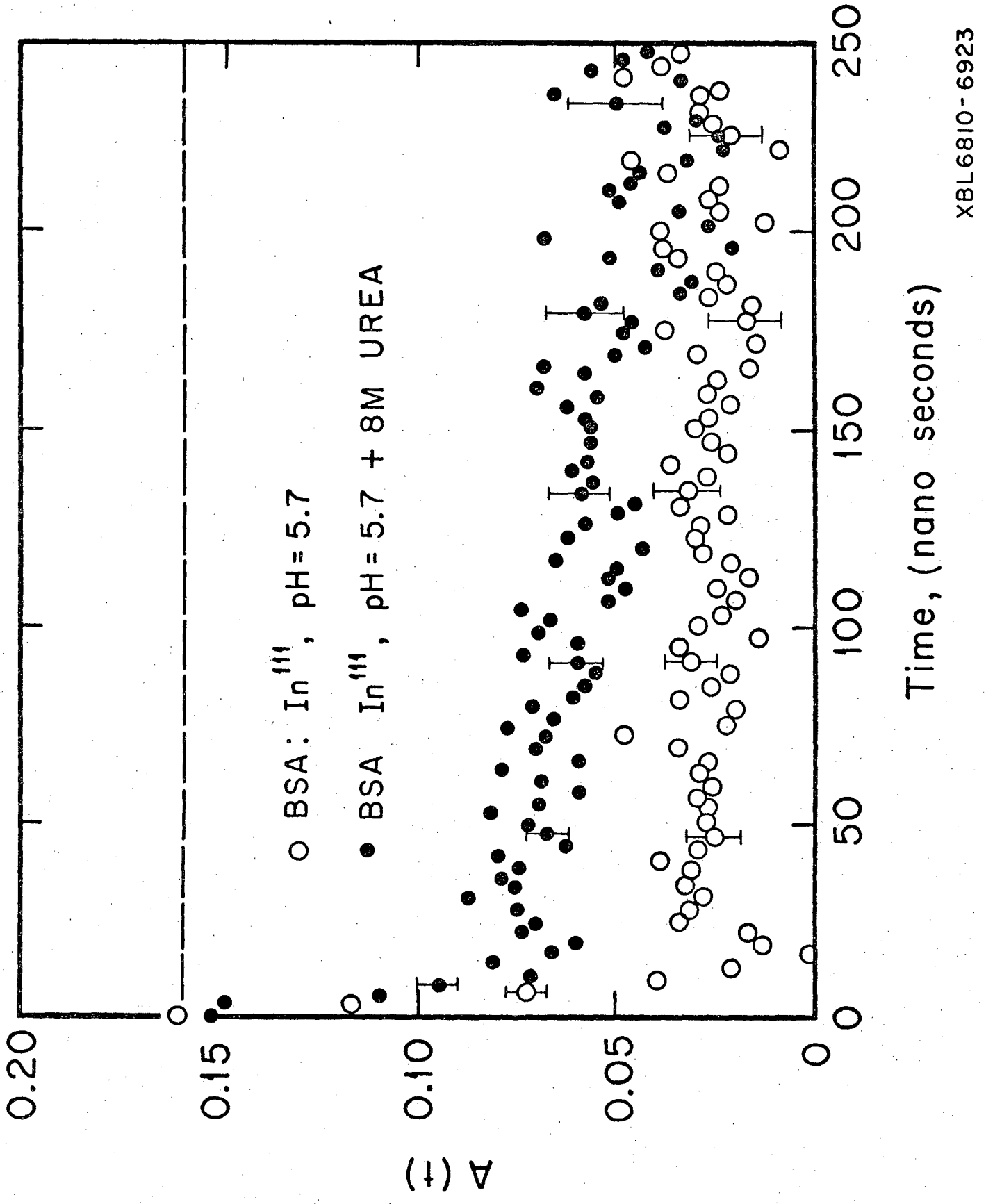
A (t)
0.20
0.15
0.10
0.05
0
0
50
100
150
200
250
Time, (nano seconds)
○ BSA: In^{111}, pH = 5.7
● BSA In^{111}, pH = 5.7 + 8M UREA
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Fig. 3