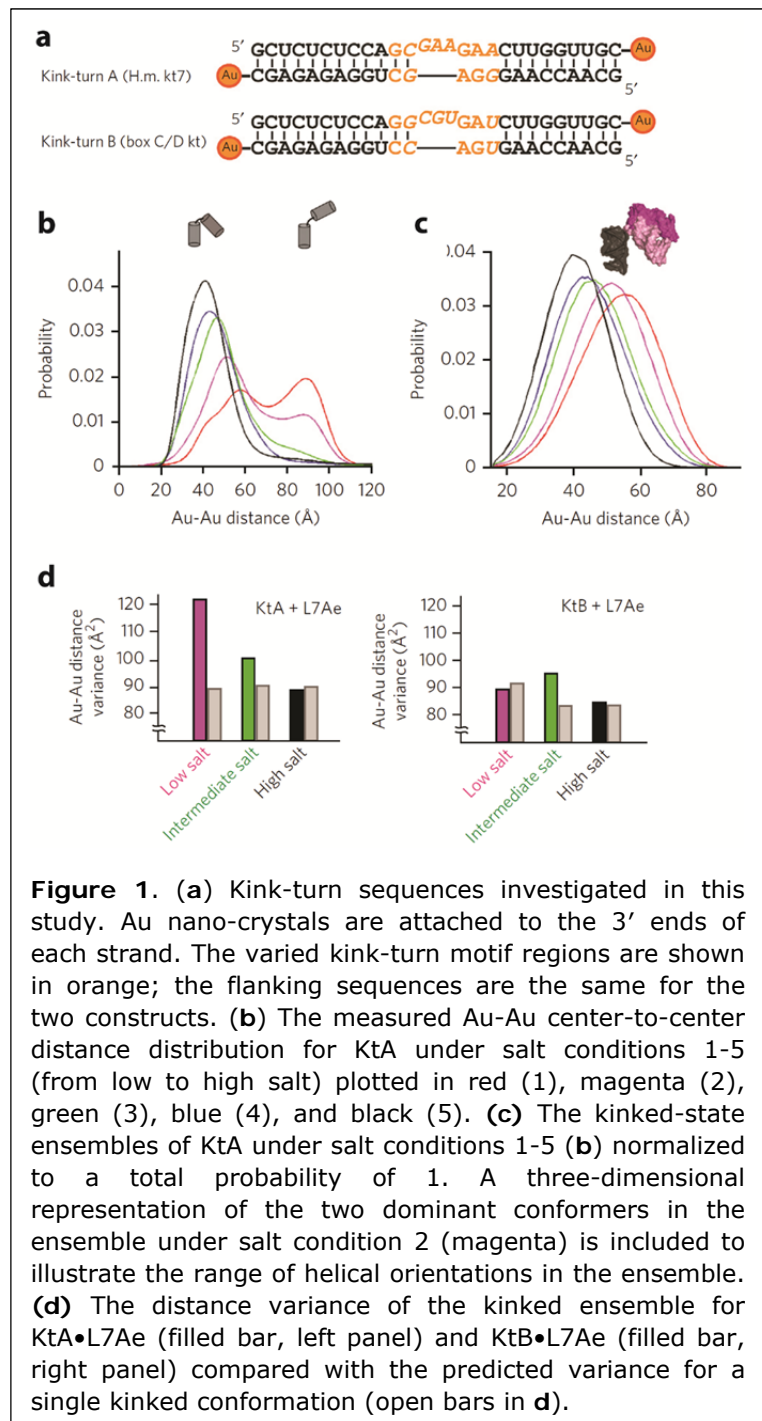




The Solution Structural Ensembles of RNA and RNA-Protein Complexes

RNAs play many functional roles in biology, from non-coding RNAs directly regulating gene expressions to structured RNAs acting as molecular machines essential to chromosome maintenance, alternative pre-mRNA splicing, protein synthesis, and protein export. RNA function typically involves a series of conformational steps, which can be considered as different conformational states that can be adopted by the same RNA. Different conformational states of a RNA can coexist in solution to form an ensemble and the ensemble can also be dominated by a single state, such as an RNA fold that is stabilized by a set of RNA structural motifs and/or bound protein. To understand RNA and RNA-mediated processes, it is necessary to uncover the ensemble nature of RNA, starting with distinguishing the co-existing unfolded and folded RNA conformational states. The next level question addresses the ensemble nature of the unfolded and folded RNA states, i.e., do they contain a single structure or ensembles of multiple structures? Little is yet known about the ensemble nature of folded RNAs in solution and still less about RNAs within RNP complexes.

To unveil the ensemble nature of RNA in solution, a task unsuited by traditional structural biology techniques, Stanford researchers, Xuesong Shi and Daniel Herschlag, turned to an emerging technique called x-ray scattering interferometry (XSI), invented and developed by Pehr Harbury and colleagues [1-3]. XSI measures the interference signals between a pair of gold nanocrystal sites spatially labeled onto a macromolecule. Related mathematically by a Fourier transformation, the interference signals report the Au-Au distance distribution, which reflects the conformational distribution of the macromolecule [1-3]. In collaboration with scientists in Dundee, they investigated the ensemble nature of a recurring RNA motif, the kink-turn, typically consisting of a three-nucleotide bulge flanked by a GA/AG tandem base pair (Fig. 1a), which stabilizes a kink of more than 90 degrees between the two flanking helices. With Au



nanocrystals attached to the ends of the flanking helices (Fig. 1a), the reporting Au-Au distance is expected to decrease as the RNA bends.

The researchers used XSI data sets collected at the SSRL Beam Line 4-2 to determine the structural ensemble for two kink-turn motifs, KtA and KtB (Fig. 1a), with and without the kink-turn binding L7Ae protein across a range of solution conditions. The basic composition of the kink-turn ensemble consists of a kinked state and an unkinked state, corresponds to gold-gold distances smaller and larger than about 60 Å, respectively (Fig. 1b). The equilibrium between the kinked and unkinked RNA is dependent on the ionic condition and differs between KtA and KtB (Fig. 1b).

The XSI data also reveal the ensemble nature of the stabilized RNA fold, the kinked-state. For the kinked or 'folded' KtA, the mean distance changes from ~53 Å to ~41 Å, suggesting that there are multiple and different kinked conformers (Fig. 1c). The larger variance of the kink-turn RNA, relative to the predicted variance for a single kink-turn conformer, provides evidence for the kinked state being an ensemble of multiple kinked conformation at all but the highest salt conditions, whereas the observed variance and predicted variances for a single kinked conformer are indistinguishable. In the simplest model, the ensemble of the kinked state of KtA contains mainly two types of kinked conformations (Fig. 1c, cartoon), one around 40-45 Å and the other around 55 Å, with varying occupancies of the conformers in these regions under different salt conditions.

The XSI data showed that addition of the L7Ae protein strongly promoted kinked states for both KtA and KtB, consistent with prior results. But what could not be seen before was that the kinked KtA·L7Ae remains an ensemble, under all but the highest ionic conditions (Fig. 1d, left). The ensemble nature of the protein stabilized kinked state differs between KtA and KtB, indicating that the protein does not fully determine the RNA's conformational preferences. Unlike the progressive narrowing in variance with increasing ionic screening for KtA·L7Ae, KtB·L7Ae appears to have narrow variance under low and high salt concentrations and a broader distance distribution at intermediate ionic conditions, presumably reflecting a mixture of the high and low salt states (Fig. 1d).

This work is the first application of XSI on RNA and RNA·protein complexes. By establishing the ensemble nature of folded RNA and RNA·protein motifs, this work sets the stage for further investigating the biological roles of ensemble properties for these and other RNAs and RNA/protein complexes and for unraveling the physical and energetic bases for complex biological processes carried out by RNA/protein complexes.

References

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Contacts

Xuesong Shi, Pehr Harbury and Daniel Herschlag, Stanford University

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