The depletion attraction: an underappreciated force driving cellular organization

Davide Marenduzzo,1 Kieran Finan,2 and Peter R. Cook2

1School of Physics, University of Edinburgh, Edinburgh, EH9 3JZ, Scotland, UK
2Sir William Dunn School of Pathology, University of Oxford, Oxford, OX1 3RE, England, UK

Cellular structures are shaped by hydrogen and ionic bonds, plus van der Waals and hydrophobic forces. In cells crowded with macromolecules, a little-known and distinct force—the “depletion attraction”—also acts. We review evidence that this force assists in the assembly of a wide range of cellular structures, ranging from the cytoskeleton to chromatin loops and whole chromosomes.

The depletion attraction

As biologists, we are all aware that ionic and hydrogen bonds, plus van der Waals and hydrophobic forces, act within and between macromolecules to shape the final structure. However, a distinct interaction, known as the “depletion attraction,” may also play a substantial role (Asakura and Oosawa, 1958; Yodh et al., 2001). This force is only seen in crowded environments like those found in cells, where 20–30% of the volume is occupied by soluble proteins and other macromolecules (Ellis, 2001; Minton, 2001, 2006). Crowding increases effective concentrations, which has important consequences (Box 1), but it also creates a force apparently out of nothing. We argue that this force drives the assembly of many large structures in cells.

Consider Fig. 1 A, where many small and a few large spheres are contained in a box, representing the many small, crowding macromolecules and the fewer, larger complexes in a cell. In physicists’ terminology, both types of spheres are “hard” and “noninteracting,” so that none of the forces familiar to biologists act between them. The small spheres bombard the large ones from all sides (arrows). When two large spheres approach one another, the small ones are excluded from the volume between the two. Therefore, the small ones exert an unopposed force equivalent to their osmotic pressure on opposite sides of the two large ones to keep them together. This osmotic effect depends on the volume that is inaccessible to the small spheres; if the small spheres could gain access to this (depleted) volume, they would force the two large ones apart. Fig. 1 B gives an alternative view. The centers of mass of the small spheres can access the yellow volume, but not the gray volumes, around each large sphere or abutting the wall. When one large sphere approaches another, these excluded volumes overlap; as a result, the small spheres can now access a greater volume. The resulting increase in entropy of the many small spheres generates a depletion attraction between the large spheres. At first glance, this seems like an oxymoron; entropy usually destroys the order that an attraction creates. But if we consider the whole system (not just the large spheres), the excluded volume is minimized and thus entropy is maximized (because there are so many small spheres).

The Asakura–Oosawa theory (“AO theory”; Asakura and Oosawa, 1958), allows us to estimate the scale of this depletion attraction (Box 1). In cells, the diameters of the large spheres are the major determinants, as the other variables in the equation in Box 1 are constant; larger spheres tend to cluster more than smaller ones (Fig. 2 A, compare i with ii). The attraction can easily be recognized in vitro; adding an inert crowding agent like a dextran or polyethylene glycol (PEG) promotes aggregation (by increasing the volume fraction, n, of the small spheres). However, the force has a maximum range of only ~5 nm, which is the diameter of a typical crowding protein; it will be larger if the two large objects fit snugly together (or are “soft” enough to fuse into one with conservation of volume) and smaller if surface irregularities limit close contact (Marenduzzo et al., 2006).

In what follows, free energy is expressed in $k_B T$ units; 1 $k_B T$ is ~0.7 kcal/mol, which is roughly comparable to the energy associated with one hydrogen bond in a protein (Pace et al., 1996). Therefore, attractions of only a few $k_B T$ are within the range that biologists know can stabilize a structure.

A simple case: actin dimerization and bundling

It is widely believed that ATP hydrolysis provides most of the energy that drives actin dimerization. However, calculation shows the depletion attraction makes some contribution, ~0.5 $k_B T$ (Fig. 2 A, i; Marenduzzo et al., 2006) compared with the experimentally determined free energy change of 1–2 $k_B T$ (Sept and McCammon, 2001; Dickinson et al., 2004). The attraction is nonspecific in the sense that it can bring two large spheres together, but it cannot orient them. Therefore, the addition of
Box 1. AO and related theories

The physics of an aqueous solution crowded with ions and macromolecules of different sizes is complicated, and various theories provide different perspectives on the underlying problems (Lebowitz et al., 1965; Ogston, 1970; Cotter, 1974; Mao et al., 1995; Minton, 1998; Parsegian et al., 2000; Kinjo and Takada, 2002; Spitzer and Poolman, 2005). The AO theory (Asakura and Oosawa, 1958) is one approximation, that shows that

$$\Delta F_{\text{gain}} = -\left[1 + 3/2(D/d)\right]nk_BT,$$

where $\Delta F_{\text{gain}}$ is the free energy gained when the two large spheres in Fig. 1 come into contact, $D$ and $d$ are the diameters of the large and small spheres, $n$ the volume occupied by the small spheres, $k_B$ the Boltzmann constant, and $T$ is the absolute temperature. This equation applies generally because particles of all sizes possess a hard core; it also applies to values of $n$ up to $\sim 0.3$, after which it becomes less accurate (Gotzelmann et al., 1998). In cells, $n$ can be determined in various ways (i.e., by cell fractionation, electron microscopy, or gel filtration), and is (luckily) between 0.2–0.3 (Busch and Daskal, 1977; Zimmerman and Trach, 1991; Bohrmann et al., 1993). $D$ thus determines the scale of the attraction (as $d$, $n$, and $T$ are usually constant). Results obtained using “molecular tweezers” show the equation to be so accurate that it is being used to position particles within manmade nanostructures (Yodh et al., 2001).

We now consider how AO theory differs from two related theories. First, both the depletion attraction and hydrophobic effect (Chandler, 2002) tend to minimize the surface exposed to the macromolecular solute or water. They are also superficially similar in that one is purely, and the other mainly, driven by entropic effects. However, an increase in volume available to a macromolecular solute drives the depletion attraction, whereas an increase in hydrogen-bonding states available to water underlies the hydrophobic effect (Chandler, 2002). The second theory is known as “macromolecular crowding” in the biological literature. “Crowding” increases thermodynamic activities, and has been successfully used to compute effects on chemical reactions and equilibria (Ellis, 2001; Minton, 2001, 2006). Macromolecular crowding describes the same phenomenon as AO theory, but is based on scaled particle theory and so cannot be applied to the (concave) structures we consider (i.e., two touching large spheres; Minton, 1998). But if the large spheres are allowed to fuse to give one larger (convex) sphere, it then gives roughly equivalent results (unpublished data). Therefore, the hydrophobic effect differs in mechanism, and macromolecular crowding differs in technical treatment.

Figure 1. The depletion attraction and its role in cellular organization. (A) Many small spheres (purple) representing soluble macromolecules bombard three large spheres (red), representing cellular complexes, from all sides (arrows). When two large spheres come into contact (right), the small ones exert a force equivalent to their osmotic pressure on opposite sides of the two large ones to keep them together. (B) The shaded regions in this alternative view show regions inaccessible to the centers of mass of the small spheres. When one large sphere contacts another, their excluded volumes overlap to increase the volume available to the small spheres (increasing their entropy); then aggregation of the large spheres paradoxically increases the entropy of the system. An analogous effect is found when a large sphere contacts the wall.

Secondary structures, tertiary structures, and helices

Within a protein, the scale of the attraction is small relative to hydrodynamic bonding. For example, forming a linear tube into a helix generates an overlap volume (Fig. 2 C, iv) so the attraction can stabilize a helix (Maritan et al., 2000; Snir and Kamien, 2005). But in the case of an $\alpha$ helix (with four hydrogen bonds per helical turn), it contributes only $\sim 0.07 k_BT$ per turn (calculated using a helix with a 0.25-nm radius and 0.54-nm pitch, and assuming $d = 5$ nm and $n = 0.2$; unpublished data). The attraction created by folding a tube into a $\beta$-sheet (to produce two cylinders lying side-by-side, as in Fig. 2 A, iii), where each amino acid makes two hydrogen bonds and strands are 0.35 nm apart, is similarly small (i.e., $< 0.02 k_BT$ per amino acid; not depicted). This is consistent with experimental observations and calculations showing that crowding agents increase the rates of refolding of lysozyme and the $\beta$-sheet WW domain by two- to fivefold (van den Berg et al., 2000; Cheung et al., 2005).
The attraction also contributes ~0.8 $k_b T$ per 14-nm turn in a coiled coil (calculated using two 0.5-nm cylinders; unpublished data), and <1 $k_b T$ per 10 bp of DNA (not depicted). Again, this is consistent with crowding agents slightly increasing the melting temperature of DNA (Woolley and Wills, 1985; Goobes et al., 2003).

### Abnormal interactions: sickle cell hemoglobin and amyloid fibrils

In larger structures, the attraction becomes more prominent. For example, sickle cell hemoglobin results from the substitution of valine for glutamic acid at the β6 site of hemoglobin; this drives end-to-end polymerization of deoxygenated hemoglobin into fibers, followed by side-by-side “zippering” into bundles. As a result, red blood cells become more rigid and so pass less rapidly through capillaries, reducing oxygen exchange and causing sickle cell anemia. As with actin, the attraction contributes slightly to dimerization (Fig. 1 C, i), but contributes many tens of $k_b T$ per micrometer of fiber length to bundling (Fig. 1 C, iii; Jones et al., 2003). It may similarly drive aggregation in many other pathologies (e.g., into amyloid fibrils in Alzheimer’s, type 2 diabetes, and the transmissible spongiform encephalopathies; Hatters et al., 2002; Ellis and Minton, 2006). As tissue hydration falls slightly on ageing (Barber et al., 1995), this may increase the volume fraction, $n$, and promote aggregation, which is consistent with the increased incidence seen with age.

### Large nuclear bodies and membrane-bound structures

Nucleoli and promyelocytic leukemia bodies disassemble when nuclei from human hematopoietic cells are immersed in a low concentration of monovalent cations; both reassemble (and nucleolar transcription recovers) when a crowding agent like PEG is added (Rosania and Swanson, 1995; Hancock, 2004). This points to a role for crowding, perhaps acting through cooperative effects and the depletion attraction (Fig. 1 C, i). If so, the
attraction could also shape other large nuclear structures, such as
splicing speckles and Cajal bodies (Spector, 2003). PEG is also
used routinely to induce cell fusion during hybridoma produc-
tion, and the attraction drives the first step, which is cell aggre-
gation (Kuhl et al., 1996; Chu et al., 2005); it also induces thylakoid
membranes to stack (Kim et al., 2005). Thus, thermodynamics
could give direction to vesicular traffic—to ward clustering
(through the attraction) and membrane fusion (by minimizing
surface curvature).

Genome looping
There are entropic costs associated with forming DNA or chro-
matin into a loop, but these can be overcome if large enough
complexes are bound to the template (Fig. 2 B, i; Marenduzzo
et al., 2006). Consider two transcription complexes; each might
contain a multisubunit polymerase, the transcript and its neu-
tralizing proteins, plus associated ribosomes (in bacteria) or
spliceosome (in eukaryotes). When they come into contact, the
resulting attraction will keep them together, thus looping the
intervening DNA. A cost/benefit analysis of the energies involved
enabled correct prediction of various types of organization.
First, looping should depend on ongoing transcription (as only
then is the complex associated with the template); it does. For
example, loops are present in all transcriptionally active cells
examined (from bacteria to man), but not in inactive ones like
chicken erythrocytes and human sperm (Jackson et al., 1984;
Cook, 2002). And as chicken erythroblasts mature into eryth-
rocytes, transcription falls progressively as loops are lost, until
no activity or loops remain (Cook and Brazell, 1976). Recent
evidence also shows that loops detected using “chromosome
conformation capture” are tied through active polymerizing
complexes (Cook, 2003). Thus, the Hbb-b1 (β-globin) gene
lies tens of kilobase pairs away, on chromosome 7, from its
locus control region, and ~25 Mbp away from a gene (Eraf)
encoding the α-globin–stabilizing protein; it contacts the locus
control region and Eraf in erythroid nuclei (where all three are
transcribed), but not in brain nuclei (where all are inactive;
Osborne et al., 2004). Second, active polymerases cluster, as
predicted. Thus, in higher eukaryotes, ~8 active polymerase
II units cluster into nucleoplasmic “factories” (Cook, 1999;
Faro-Trindade and Cook, 2006), and bacterial ribosomal DNA
operons aggregate similarly (Cabrera and Jin, 2003). Active
DNA-polymerizing complexes in both pro- and eukaryotes
also cluster into analogous replication factories (Cook, 1999),
and the bacterial ones separate (Bates and Kleckner, 2005) just
when the looping cost exceeds the attraction. In all cases, the
scale of the attraction relative to the looping cost correlates with
the clustering seen.

Conclusions
We have argued that an osmotic depletion attraction drives the
organization of many cellular structures. Unlike other noncova-
lent interactions (i.e., ionic and hydrogen bonds, van der Waals
and hydrophobic forces), this one only becomes significant in
crowded environments like those in cells. It is nonspecific in the
sense that it can bring spheres together without orienting them.
It also depends on size and shape; the larger the overlap
volume, the larger the attraction. Just as the entropy of the sol-
vent (water) mainly underlies the hydrophobic effect, that of the
solute (the crowding macromolecules) creates the attraction.
These generalizations come with caveats because the under-
lying physics is complicated, and AO theory involves several
simplifications (e.g., it becomes less accurate when \( n \) is >0.3,
and it takes no account of kinetics). Nevertheless, the concept of
a hydrophobic force is useful to biologists despite the under-
lying complexity, and we believe the concept discussed in this
work will be similarly useful, especially because its scale can be
calculated so simply.

Many questions remain. On the theoretical side, what
happens when \( n \) increases above 0.3, and the AO equation
becomes less precise and the theory much more complicated
(Gotzelmann et al., 1998)? What are the relative advantages
and disadvantages of the different theories of crowded solu-
tions (Box 1)? On the experimental side, what exactly is the
volume fraction within a cell, and how closely can typical pro-
teins approach each other? Could the attraction help nucleo-
somes strung along DNA pack into the chromatin fiber (Fig.
2 B, ii). Can clumps of heterochromatin be treated as spheres
that are subject to the attraction? If so, the attraction could un-
derpin the condensation of an (interphase) string of such clumps
into the mitotic chromosome (Fig. 2 B, ii; Manders et al., 1999).
Could it also underpin the pairing of chromosomes seen dur-
ing meiosis and polytenization, where a string bearing a unique
array of factories and heterochromatic clumps aligns in perfect
register with a homologue, but not with others carrying differ-
ent arrays (Fig. 2 B, iii; Cook, 1997)? Could it drive end-to-end
pairing of chromosomes? For example, diploid human lympho-
cytes contain 10 chromosomes encoding nucleolar organizing
regions (NORs), but only ~6 NORs are transcribed, and only
these aggregate to form nucleoli (Wachtler et al., 1986). Does
the attraction act through the thousands of active polymerizing
complexes associated with each active NOR to drive nucleolar
assembly (Fig. 2 B, iv)? Could it similarly drive the cluster-
ing of heterochromatic centromeres into chromocenters (Fig.
2 B, iv)? We have also seen how the attraction contributes to
protein folding, but what of the special case where a protein is
so confined that the overlap volume resulting from contact with
the surrounding wall becomes significant (Fig. 2 C)? Do pores,
and the barrels formed by chaperonins, proteasomes, and exo-
somes (Lorentzen and Conti, 2006), all exploit the attraction to
promote ingress of their target proteins (Martin, 2004; Cheung
et al., 2005; see Ellis, 2006, for a review of how crowding af-
fects protein folding in confined spaces)? Clearly, we need to
extend the experimental studies on the simple model systems
reviewed in this study to complex subcellular assemblies, much
as Hancock (2004) describes.

As soon as cellular structures become larger than ~75 nm,
the overlap volume can generate an attraction of ~5 \( k_B T \); this
is probably sufficient to promote irreversible aggregation when
cooperative effects are included (Fig. 2 A, i, inset; Marenduzzo
et al., 2006). This begs the obvious question: why don’t all
large structures in the cell end up in one aggregate (just as
overexpressed bacterial proteins form inclusion bodies)? We
suggest they will tend to do so unless energy is spent to stop
aggregation and/or inert mechanisms prevent it. For example, anchorage to a larger structure (e.g., the cytoskeleton), surface irregularities (Jones et al., 2003), or charge interactions could all prevent close contact, and thus reduce the attraction. All seem to operate; for example, >70% of proteins in Escherichia coli and Bacillus subtilis (and >90% of the most abundant ones) are anionic at cellular pH, and thus would be expected to repel each other (Eymann et al., 2004; Weiller et al., 2004). We also note that structures like the cytoskeleton and membrane-bound vesicles are not rigid and permanent; rather, they continually turn over, to reduce their effective size and ensure that a large structure does not persist long enough to aggregate (Misteli, 2001; Altan-Bonnet et al., 2004). Nature, although constrained by the second law of thermodynamics, finds ways around it.

We thank the Biotechnology and Biological Sciences Research Council, the Engineering and Physical Sciences Research Council, Cancer Research UK, the Medical Research Council, and the Wellcome Trust for support. K. Finan is supported by the E.P. Abraham Trust, a Clarendon Fund award from the University of Oxford, and an Overseas Research Student award from the UK government.

Submitted: 11 September 2006
Accepted: 24 October 2006

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