Interactions in systems with short-range attractions and applications to protein crystallisation

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Abstract. The problem of phase behaviour of solutions of globular proteins is approached by means of a Hard Core Yukawa fluid model with short-ranged attractions. We have determined the phase behaviour of this model system for different well widths, using a variety of high quality methods. The essential phase behaviour of systems with short-ranged attractions is reproduced. The typical phase behaviour of solutions of globular proteins is well represented by the Hard Core Yukawa fluid with short-ranged attractions. The formation of amorphous precipitates in protein crystal growth experiments is identified on our model phase diagram with the formation of an attractive glass. As the range of attraction is decreased, the formation of an attractive glass state dominates the phase behaviour in the meta-stable regime above the critical point. We show how the addition of a long-ranged attraction, in the Two-Yukawa model, has the effect of eliminating the formation of attractive glass, while preserving the equilibrium features of the short-ranged attractive system. This opens the possibility of using attractive long-range interactions to avoid the formation of an attractive glass state.

1. Introduction

In recent years, rapid developments in Molecular Biology now mean that one can synthesise any protein relatively cheaply and easily. However, although the sequence of such a protein - natural or synthetic - can be determined (or in the case of synthesis pre-determined), obtaining the structure of it's native or folded state is a far less trivial matter. It has been shown by various techniques including NMR studies that the crystallised protein has in general the same structure as the solute protein [1].

At present the most common technique for this investigation is X-Ray Crystallography of the folded protein. At present, the structures of less than 10% of known proteins have been determined. The bottleneck in this process is the difficulty of producing good quality crystals of proteins in their native state. We attempt to model globular proteins, apparently the simplest case.

One of the most difficult aspects of this field is the many varied experimental observations associated with attempts, successful or otherwise, to crystallise proteins. These include the presence of a "cloud-point" which is meta-stable with respect to the equilibrium solubility curve [2], Hofmeister's empirical series to determine which metal ion to use in 'salting out' crystals [3, 4], enhancement of crystallisation by addition of different molecular weight polymers [5, 6, 7], a strong tendency to form amorphous gel-like solid structures, often at surprisingly low density, which can inhibit [8] or prevent crystal growth [2].

A system consisting of relatively large solute particles dissolved in a solvent behaves analogously to a monatomic substance (eg. a noble gas). There are distinctive phases of very low concentration (gas) and very high concentration (liquid) with fluid-like structure, as well as a solid regular array (crystalline) phase. Liquid-gas coexistence and a critical point as well as solid-liquid coexistence are observed. The simplest such system is the so-called "hard-sphere" system, consisting of rigid particles which only interact with each other on contact. Here, only two phases are observed: crystal and fluid. Only one distinct fluid phase exists. This reflects the driving force for liquid-gas phase separation. If one considers a similar system with an added attraction at high temperature, the same homogeneous fluid exists. As one lowers the temperature however, the attractive force becomes more important. A situation then occurs where the lowest free energy for the system may be the gas phase driven by optimisation of the entropy (for low densities), exclusively the liquid phase driven by optimisation of enthalpy of attractions (for high densities), or a two phase liquid-gas coexistence that optimises the free energy by separately maximising the entropy (gas) and minimising the enthalpy (liquid) contributions to the free energy. The critical point is the first sign of incipient coexistence, a single point on the temperature-density phase diagram where the liquid and gas phases have identical chemical potential and pressure.

In the case of and proteins, X-Ray scattering experiments have shown that the range of the attraction is small relative to the size of the particle (for lysozyme on the order of 8% [9]). Such short-ranged attractive systems, which we will discuss here, display markedly different characteristics to the usual gas (dilute-solution), liquid (concentrated solution) and crystal systems.

With this in mind, we propose a simple model of hard spheres interacting through a short ranged Yukawa potential, which can reproduce the main features of typical protein phase diagrams [10]. We and others have recently shown that one of the most prominent features of short-ranged attractive systems, independent of the details of the potential, is attractive glass formation [11, 12, 13, 14]. Glasses (solids with a liquid-like structure) have traditionally formed at high density driven purely by close packed repulsions. This state we call the "repulsive glass". However, when the inter-particle attractive potential is narrow (as it is in our model) a new type of "attractive glass" may form at lower density. The lifetime of the attractive glass is typically such that crystal growth may not occur over experimental time-scales.

Naturally, the attractive glass only forms at low temperatures, where the thermal energy of the particles is small compared to the attractive well. One point is that, in addition, such a glass forms more easily when the range of the potential narrows. However, proteins denature at even moderately high temperature, so a pertinent question is whether one can reach a high enough temperature to avoid the glass without denaturing the protein.

We first discuss the methods we have used to compute the phase diagrams. We then calculate the equilibrium phase diagrams for different well widths and overlay the glass lines, showing at what temperature and density the glass forms. We aim to identify the different widths of attraction for which protein crystallisation may be feasible.

2. Methods

The Hard Core Yukawa fluid is described by the following inter-particle potential

$$v(r) = \begin{cases} \infty & r < \sigma \\ -A_0 \frac{e^{-b(r-\sigma)}}{r/\sigma} & r \geq \sigma \end{cases} \tag{1}$$

The parameter b determines the range of the interaction- larger b values correspond to shorter ranges of attraction. The parameter A_0 defines the energy scale. In this paper we set the particle diameter $\sigma = 1$ and $A_0 = 1$, so that the range b is in units of hard core diameter and the temperature is in units of energy.

We computed the phase diagram for the Hard Core Yukawa fluid using a variety of methods. We use a self consistent approximation described below to calculate the properties of the liquid, gas and fluid phases, and perturbation theory for the crystal phase, locating the phase boundaries by imposing the standard conditions on the chemical potential μ and pressure P,

$$\mu_{solid} = \mu_{fluid} \tag{2}$$

$$P_{solid} = P_{fluid} \tag{3}$$

The glass lines, as calculated from Mode Coupling Theory, are overlaid on the phase diagram. We now briefly describe the methods of the Self Consistent approximation, perturbation theory and Mode Coupling Theory.

2.1. Self Consistent Ornstein Zernike Approximation (SCOZA)

The Ornstein-Zernike (OZ) equation for the pair correlation function $h(r)$ is

$$h(r) = c(r) + \rho \int d\mathbf{r}' c(|\mathbf{r} - \mathbf{r}'|) h(|\mathbf{r}'|) \tag{4}$$

where $g(r) = h(r) + 1$ is the radial distribution function and $c(r)$ the direct correlation function [15].

The Self Consistent Ornstein-Zernike Approximation (SCOZA) is designed to provide a closure to the OZ equation. Various approximations exist (eg. mean spherical approximation, Percus-Yevick approximation, hyper-netted chain approximation etc.) which relate the direct correlation function $c(r)$ to the potential with one or more state dependant parameters that can be adjusted to satisfy various exact thermodynamic relations [16].

The Self Consistent approximation alluded to here uses a Yukawa function to account for the contribution to the direct correlation function $c(r)$ due to the hard-core repulsion. Two thermodynamic conditions are imposed: one requires that the compressibility and the virial route to the thermodynamics lead to the same system properties- namely the Carnahan-Starling equation of state for the hard-sphere fluid; the other condition requires that the energy and compressibility routes yield the same result. These two conditions provide a means to calculate the thermodynamics in a self-consistent manner. Using the Yukawa interaction potential we can establish relations that allow us to compute all the thermodynamic properties of the system [16, 17].

The application of SCOZA to an attractive hard-core Yukawa fluid provides a semi-analytic calculation of the thermodynamic properties of the fluid, liquid and gas states that arise in systems interacting through short-range forces [16]. SCOZA has been applied to Yukawa systems with relatively large values of the range of the potential, with a satisfactory reproduction of the liquid-vapour binodal curves and a good description of the critical point region. The accuracy of SCOZA is maintained at high density and low temperature, in contrast to other methods. In fact, the agreement of SCOZA for narrower wells with recent Gibbs Ensemble Monte-Carlo simulations [18] are very good [19], giving us confidence in the accuracy of the phase diagrams calculated by the SCOZA method for the well widths that we present.

2.2. Perturbation Theory

Perturbation Theory is employed to calculate the phase behaviour of the crystalline phase following the lead of Gast *et al.* [20, 21]. At the simplest level this theory makes the assumption that one can divide the free energy of the system up into the contribution caused by the hard core part of the potential and a perturbation composed of an attractive tail as follows,

$$v(r) = v_0(r) + v_{att}(r) \tag{5}$$

The hard core being the greatest contribution to the potential is the zero order or "reference" free energy. The attractive tail is the perturbation. This amounts to a series of corrections to the reference where each subsequent term in the series is hopefully smaller than the previous one. Unfortunately even to calculate the second order term in this expansion is extremely demanding. However, Barker and Henderson [22] have developed a very accurate approximation to the second order term which we have used:

$$\frac{\beta F}{N} = \frac{\beta F_0}{N} + \frac{\beta\rho}{2}\int v_{att}(r)g_0(r)d\mathbf{r} - \frac{\beta\rho}{4}\left(\frac{\partial\rho}{\partial P}\right)_0 \int v_{att}^2(r)g_0(r)d\mathbf{r} \tag{6}$$

The free energy of the reference system is calculated by thermodynamic integration in the packing fraction using an equation of state for the hard-sphere FCC solid proposed by Hall [23].

The perturbation terms were evaluated using an analytic form of the pair distribution function $g_0(r)$ for the hard-sphere FCC solid proposed by Kincaid and Weiss [24]. Once we have the Helmholtz free energy we can calculate the Gibbs free energy and hence the pressure.

For extremely narrow well widths a solid-solid transition occurs analogous to the liquid-gas binodal. The corresponding solid-solid critical point compares with quantitative accuracy to Monte-Carlo simulations of the Hard Core Yukawa fluid [25, 26]. From this evidence we are confident of this method's accuracy.

2.3. Mode Coupling Theory (MCT)

MCT describes the transition of super-cooled liquids to a non-ergodic state [27]. The transition of the super-cooled liquid to the glass state represents a critical slowing down of the particle motions, leading to structural arrest. A characteristic property of the arrested state is that it has the static structure of a liquid. Apart from the parameters describing the microscopic motion, the static structure factor $S(q)$ is the only input to MCT, which aims to give a complete description of the dynamical properties of the system.

MCT has been successfully applied to the study of certain aspects of the arrest transitions of colloidal particles [28, 29], and details of time correlation functions are well reproduced [30]. MCT is expected to provide a good description of the principal phenomena of glass formation in the regions of the phase diagram we are most interested in.

To achieve a high quality description of the arrest transition a good quality static structure factor is required. The SCOZA method we have used provides us with many thermodynamic properties of the system. We use the direct correlation function $c(r)$ as produced by SCOZA to construct the structure factor $S(q)$. The structure factor calculated in this way is expected to be highly accurate. With this structure factor input we solve the MCT equations to determine the final dynamical state of the system.

3. Results

3.1. Yukawa $b = 5.0$

The Yukawa $b = 5.0$, corresponds to an attractive range of about 12% of the particle radius, if we consider the range at half the potential depth. The phase diagram displays all the features of a conventional Van der Waals type phase diagram. Three distinct phases exist, solid, liquid and gas. At high temperatures only a single fluid and the usual solid phase exists. As the temperature is lowered past the critical point C_p, two distinct fluids become apparent, one more (liquid) and one less (gas) dense. These two phases coexist at moderate densities. As the temperature lowers further a unique triple point T_p is reached where all three phases solid, liquid and gas coexist.

With the usual equilibrium lines, we have shown also the MCT glass transition line, beyond which the system may no longer reach equilibrium. For this range of attraction, we see only the presence of a repulsive glass. The formation of this high density repulsive glass is almost entirely temperature independent. For this well width here is almost no attractive glass formation.

3.2. Yukawa $b = 30.0$

The $b = 30.0$ well corresponds to an attractive range of about 3% of the particle radius. The first thing we observe is that the critical point has become meta-stable with respect to the solubility curve. In such a situation no triple point can exist as only two equilibrium phases are ever possible. This phase diagram, typical of short-ranged attractions, is similar to that observed for the proteins γ_{II}-crystallin[31] and lysozyme[2]. We have labelled three zones I, II and III in Figure 2 in accordance with the ideas of Muschol and Rosenberger [2], and we believe the microscopic picture offered underlies the corresponding zones in their schematic phase diagram. Note that zone I is the regime where one can obtain good crystals.

On decreasing the temperature, the narrower range of attraction causes a low density, attractive glass to form. The attractive glass line lies at a higher temperature than the liquid-gas coexistence for a wide range of densities.

Changing the range of attraction clearly alters both the equilibrium phase behaviour and the tendency to glass formation. To appreciate the significance of this on the phase behaviour, consider quenching down below the solubility curve towards the critical temperature in Figure 2. It is clear that the range of temperatures in which equilibrium can be achieved is now limited by the presence of the attractive glass. This situation becomes more extreme as the well is narrowed further [19] with the attractive glass dominating at still lower densities. If the quench goes below this glass line, we expect to see the formation of various types of gel. These non-equilibrium structures may be long-lived and can thus prohibit or delay crystal formation beyond experimental time-scales. This sort of behaviour is commonly observed in protein crystal growth experiments where amorphous precipitates interfere with crystallisation.

3.3. Two-Yukawa $b_1 = 30.0, b_2 = 5.0$

As stated previously, the protein phase diagram is extremely sensitive to the addition of monovalent salts. The Hofmeister series describes the relative efficiency of different ions on protein crystal growth. Protein crystallisation can also be enhanced by the addition of various polymers such as polyethylene glycol. The addition of the polymer is associated with depletion interactions. Both of these cases show that modifying the range and nature of the interactions lead to important changes in the phase behaviour. Without investigating the origin of these interactions, we chose to study a modification of our model, by including a longer ranged attraction to our short-ranged Yukawa Hard Core fluid [32].

Starting from our previous short-ranged $b_1 = 30$ well we added the longer-ranged $b_2 = 5$ well

$$v(r) = \begin{cases} \infty & r < \sigma \\ -\frac{1}{A_1+A_2}\left[A_1\frac{e^{-b_1(r-\sigma)}}{r/\sigma} + A_2\frac{e^{-b_2(r-\sigma)}}{r/\sigma}\right] & r \geq \sigma \end{cases} \quad (7)$$

constraining the overall strength of attractions to be the same as the One-Yukawa model $A_1 + A_2 = 1$.

In Figure 3 we see that the phase behaviour displays interesting features of both short and long ranged attractive systems. Like the $b = 30$ case the critical point is meta-stable with respect to the solubility curve. Apart from a minor shift in the temperature and density, the equilibrium phase behaviour of the Two-Yukawa system is essentially the same as the One-Yukawa system.

More importantly, the attractive glass line, which dominates above the critical temperature of the $b = 30$ model, is no longer present and we see only the repulsive glass line at high density. The effect of adding a long ranged attraction is to diminish the role of glass formation in the phase behaviour. The role of long range attractions in lifting the meta-stable critical point out of the region where glass formation dominates has been investigated before [32]. This is most interesting. It implies that zone I in Figure 3 can be considerably extended by moving the glass curve.

4. Conclusions

We have presented the phase diagrams and glass lines of a model hard-core Yukawa fluid for various ranges of attraction, which we have computed using a variety of high quality methods. The resulting phase diagrams are consistent with simulated phase diagrams of the Hard Core Yukawa fluid [33] and bear striking resemblance to the reported phase diagrams of globular proteins [2, 31].

We find that when the range of attraction becomes smaller in comparison with the particle size, the attractive glass dominates an increasingly larger region of the phase diagram above the metastable critical point. The formation of the attractive glass is responsible for many of the problems associated with crystallising such systems (proteins, colloids). By decreasing the width of the well we find that the region of the phase diagram where crystals can grow is steadily reduced in favour of the formation of a glassy state. This behaviour is seen in real protein crystallisation experiments where amorphous precipitates interfere with crystal growth.

We have developed highly accurate methods for calculating the phase diagram of short-ranged attractive systems, allowing us to identify the regions of the phase diagram where the formation of the attractive glass can be avoided. It should now be possible to identify the optimal conditions for crystal growth in these systems.

The Two-Yukawa model can combine multiple interactions of varying strengths, from which we can accurately calculate phase behaviour (coexistence and glass lines).

We have studied the Two-Yukawa model as simple extension to account for long-ranged interactions. In the Two-Yukawa model we find phase behaviour similar to that of the short-ranged attractive system. However, attractive glass formation does not occur. At high densities a temperature independent repulsive glass does form, but the absence of the attractive glass at lower densities suggests a role for long-ranged attractions in enhancing crysal growth by avoiding the formation of glass state. The purpose of this work is to accurately model the phase behaviour of short-ranged attractive systems, and to extend this model to investigate the role of longer-ranged interactions, which might represent for example a depletion potential. Work is in progress to study the Two-Yukawa model in more detail.

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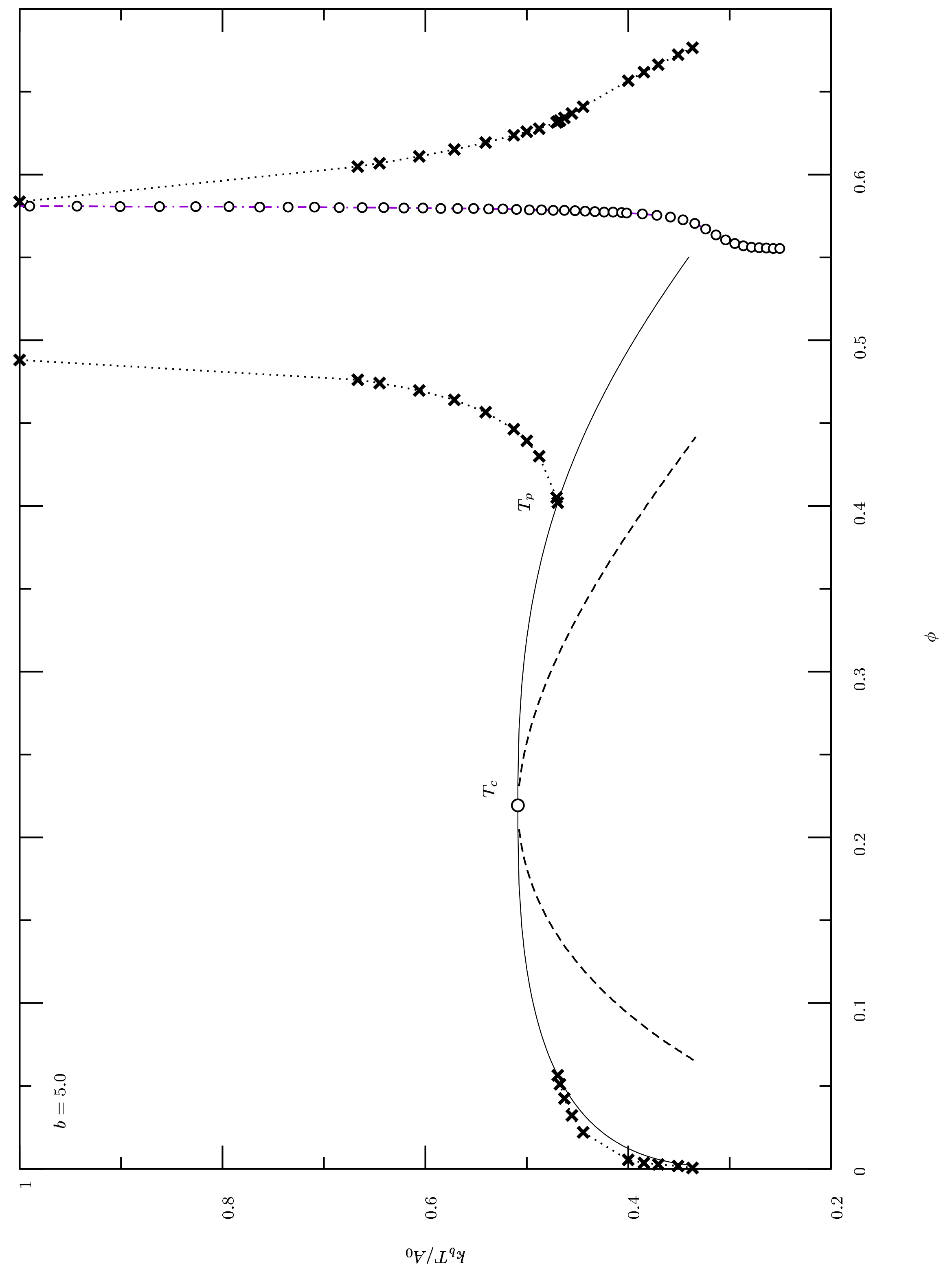

Figure 1. Yukawa $b = 5.0$. The × denotes the fluid-solid coexistence and ∘ denotes the glass line

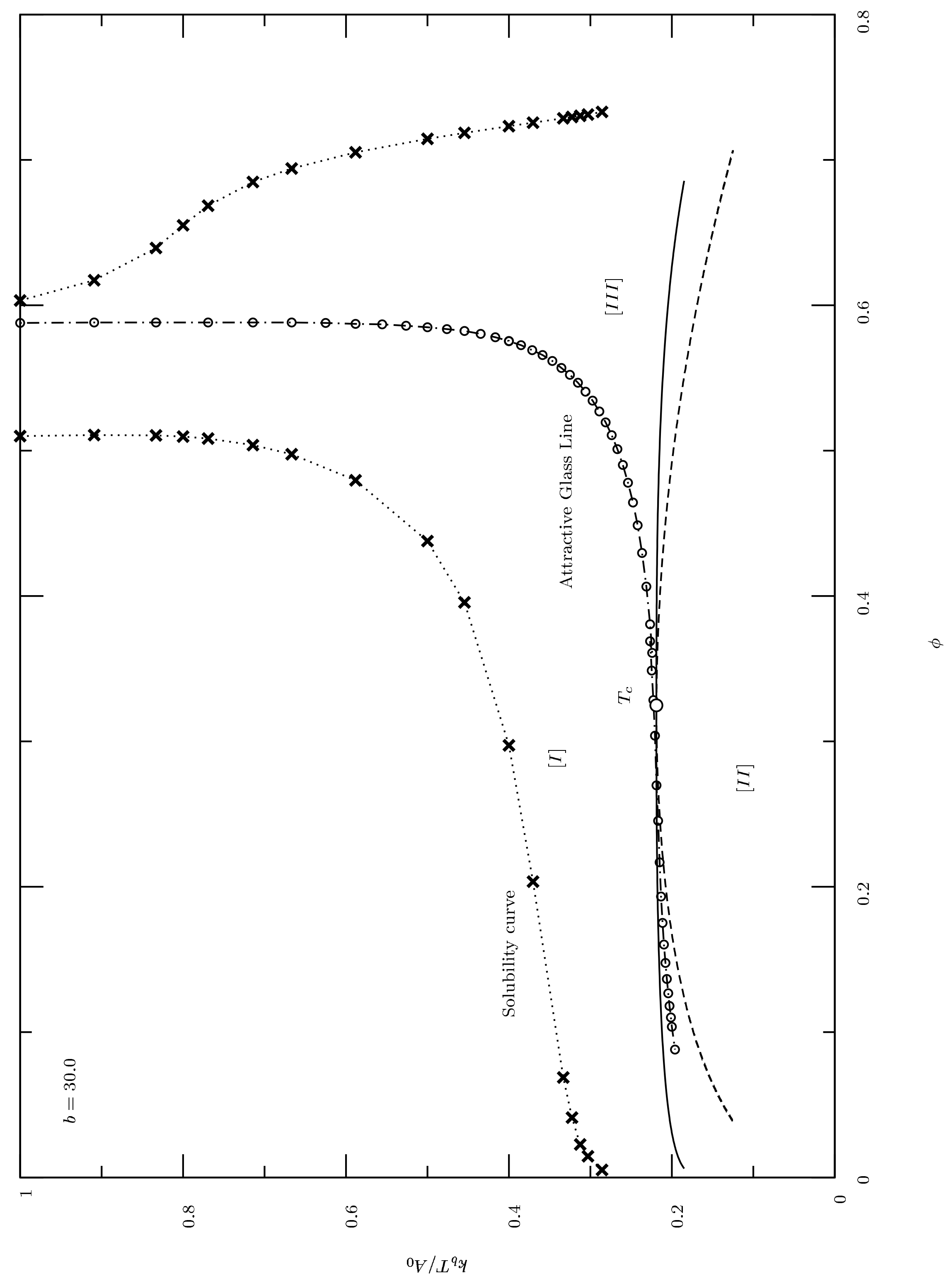

Figure 2. Yukawa $b = 30.0$. The label are the same as Figure 1. Zone *I* is the region where one can obtain good crystals. Zone *II* is the meta-stable region of fluid-fluid phase separation and zone *III* corresponds to the region of attractive glass formation. The attractive glass line has been rescaled in density from 52% so that it agrees with the actual density of the hard sphere arrest transition at 58%.

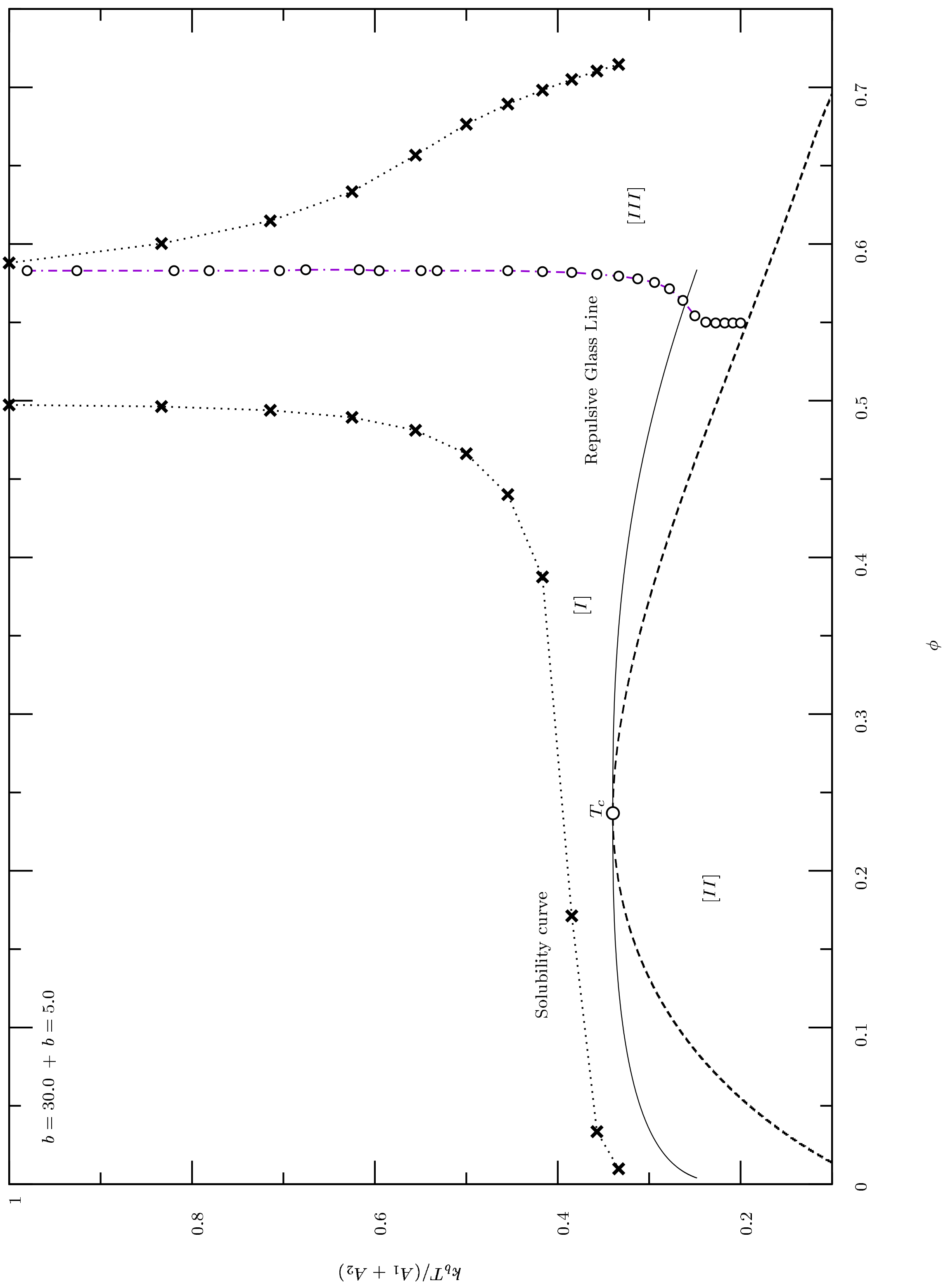

Figure 3. Yukawa $b_1 = 30.0 + b_2 = 5.0$. The labels and zones are as in Figure 2. The important difference from the $b = 30.0$ model is zone II has been considerably extended at the expense of the attractive glass (zone III).